INDEX NUMBER

Vol. 30 No. 6

U
O
JAN 7 1952
DENTISTRY
LIBRARY

Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Editor

HAMILTON B. G. ROBINSON

DECEMBER, 1951

The Editor's Viewpoint, Letters to the Editor ---------- 753

The Effects of an Acid and a Neutral Solution of Sodium Fluoride on the Incidence of Dental Caries in Young Adults. Norman H. Rickles, D.D.S., M.S., and Hermann Becks, M.D., D.D.S. (George Williams Hooper Foundation for Medical Research, San Francisco, Calif.) ---------- 757

Bacteriologic Studies of the Advancing Dentinal Lesion. George W. Burnett, D.D.S., Ph.D., and Henry W. Scherp, Ph.D. (The University of Rochester, Rochester, N. Y.) ---------- 766

Hamster Caries. David F. Mitchell, D.S. Chernausek, and Edith Zak Helman. (University of Minnesota, Minneapolis, Minn.) ---------- 778

Sympathicotony of the Salivary Glands as a Cause of Dental Caries. Werner Rathje. (Kaiser Wilhelm Institut für Biochemie, Tübingen, Germany) ---- 783

Effects of Ionizing Radiation of Oral Structures. I. Pilot Studies on Dental Caries in the White Rat. C. A. Schlack and F. Ellinger. (Naval Medical Research Institute, Bethesda, Md.) ---------- 787

A Histochemical Evaluation of Normal and Inflamed Human Gingivae. Samuel Turesky, Irving Glickman, and Tamara Litwin. (Tufts College Dental School, Boston, Mass.) ---------- 792

(Contents continued on inside back cover)

Published for THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
By The C. V. Mosby Company, St. Louis 3, Mo.

Copyright 1951 by The International Association for Dental Research

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORS OF THE JOURNAL OF DENTAL RESEARCH

Editor Emeritus—William J. Gies

Publication Committee—J. Frank Hall, Paul E. Boyle, and Hamilton B. G. Robinson.

Editor—Hamilton B. G. Robinson (Ohio State University). *Business Manager*—J. Frank Hall (Indiana University). *Circulation Manager*—Paul E. Boyle (University of Pennsylvania).

Assistants to the Editor—Paul C. Kitchin and J. B. Brown.

Board of Editors—Editor emeritus, members of the Publication Committee, and sectional representatives named in the fifth column below.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1951)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
North America	Halifax	J. S. Bagnall	J. S. Bagnall	J. S. Bagnall
	Toronto	A. W. Ellis	A. W. Ellis	A. W. Ellis
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
	Ann Arbor	Philip Jay	Philip Jay	Philip Jay
	Baltimore	M. S. Aisenberg	E. C. Dobbs	J. Ben Robinson
	Birmingham	L. Robinson	J. F. Volker	J. P. Lazansky
	Boston	P. H. Keyes	R. S. Manly	R. O. Greep
	Chicago	E. B. Tilden	G. R. Lundquist	G. B. Denton
	Cleveland	S. W. Chase	T. J. Hill	S. W. Chase
	Columbus	H. B. G. Robinson	L. F. Edwards	P. C. Kitchin
	Detroit		P. C. Lowery	
	Houston	I. R. Telford		S. S. Arnim
	Indianapolis	R. W. Phillips	J. F. Hall	M. K. Hine
	Iowa City	A. O. Klaffenbach	A. W. Bryan	G. S. Easton
	Kansas City	Ben W. Warner	H. L. Myers	N. A. Moore
	Los Angeles	L. A. Bavetta		R. L. Rutherford
	Louisville	R. E. Myers	D. Y. Burrill	D. Y. Burrill
	Minnesota	D. F. Chernausek	W. D. Armstrong	S. A. Lovestedt
	Nebraska	D. A. Keys	D. A. Keys	R. T. Ireland
	New Orleans	H. W. Peterson	H. W. Peterson	F. W. Wolfe, Sr.
	New York	Isaac Neuwrith	G. Bevelander	F. Krasnow
	Pacific Northwest	N. M. Phatak	H. J. Noyes	B. O. A. Thomas
	Philadelphia	N. B. Williams	P. E. Boyle	S. L. Rosenthal
	Pittsburgh	T. W. Brand	W. F. Swanson	E. G. Meisel
	Richmond	H. D. Coy	A. D. Brashear	O. W. Clough
	Rochester	J. W. Hein	E. R. Costich	
	San Francisco	S. Epstein	W. C. Fleming	Hermann Becks
	St. Louis	E. A. Jasper	W. B. Gurley	E. F. Allen
	Southern Calif.	L. A. Bavetta	B. Z. Rabinowitch	R. L. Rutherford
	Tennessee	J. T. Ginn	O. A. Oliver	
	Washington	J. S. Oartel	F. A. Arnold, Jr.	D. B. Scott
Not Belonging to a Division	Budapest	Ervin Lörinczy		
	Chengtu	R. G. Agnew	R. G. Agnew	R. G. Agnew
	Denmark	P. O. Pederson		
	Johannesburg	J. Staz		
	London	M. A. Rushton		
	Mexico	F. R. Leycegui	F. R. Leycegui	S. Fastlicht
	Palestine	S. Lewin-Epstein		
	Prague	F. Neumann		
	Austria, Vienna Center	F. Driak		
Dental Materials Group		R. W. Phillips	F. A. Peyton	N. O. Taylor

OFFICERS OF THE ASSOCIATION

President—L. S. Fosdick, *President-elect*—M. K. Hine, *Vice-president*—F. A. Arnold, Jr., *Secretary-Treasurer*—E. H. Hatton, *Assistant to the Secretary-Treasurer*—D. Y. Burrill, *Editor*—H. B. G. Robinson, *Trustees*—H. Trendley Dean, P. C. Kitchin, T. J. Hill, H. C. Hodge, L. M. Waugh.

Vol. 30, No. 6, December, 1951. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription price: United States, its Possessions, Pan-American Countries, $9.00 per volume: Canada and other foreign countries, $9.50. Single number when available, $1.25. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the Act of March 3, 1879. Printed in the U. S. A.

EDITOR'S VIEWPOINT

Rickles and Becks (p. 757) demonstrate that neutral sodium fluoride solution applied topically reduces caries in young adults and that acidulated fluoride solutions are ineffective, confirming other studies. This helps to guide the clinician who is asked the value of topical fluoridation in adults.

Burnett and Scherp (p. 766) found cocci, lactobacilli, and actinomycetes at the advanced region of dentinal caries' lesions. In vitro, the lactobacilli and filaments could produce enough acid to decalcify dentin but the production of adequate proteolytic enzymes by the cocci was less definite. The authors suggest further investigation of the cocci before eliminating them as agents active in the destructive process. This study was long overdue.

The hamster must be more thoroughly evaluated if it is to be used as an experimental animal in caries control studies. Mitchell, Chernausek, and Helman (p. 778) have investigated the effects of various sugar levels and simplified caries scoring methods in these animals.

Rathje (p. 783) has conducted two interesting experiments, one showing the effect of viscosity of the media on diffusion of acid production in vitro and the other showing a correlation between caries' activity and flow of saliva. Although much discussion has appeared on this subject little data is available. These investigations add to our too meagre knowledge in the field.

It has been recognized for many years now that the teeth in mouths of individuals who have received deep radiation therapy around the face and neck often disintegrate rapidly with dental caries. The mechanism of this is still unrecognized, although there have been a number of suggestions. Schlack and Ellinger (p. 787) report a pilot study on the production of a dental caries in rats by use of ionizing radiation. This experimental evidence adds to our knowledge of the effect of irradiation on the teeth and, again, suggests that teeth in the mouths of individuals who are to receive heavy irradiation around the neck and jaws should be removed before irradiation therapy.

Turesky, Glickman, and Litwin (p. 792) used histochemical methods to investigate normal and inflamed gingiva. With all relatively new research techniques care must be taken to insure proper use and even greater caution is necessary in interpretation of results. The fact that the present investigators failed to find appreciable quantity of glycogen in normal epithelium in contradistinction from the findings of others (Engel, Ray, and Orban, *J. D. Res.* **29**: 410, 1950) emphasizes the delicacy of the technique and the importance of careful evaluation. The findings on phosphatase agree with those of Zander (*J. D. Res.* **20**: 247, 1941), Vallotan (*Schweiz Monat. f Zahn.* **52**: 512, 1942), and Calabrini and Carranza (*J. D. Res.* **30**: 28, 1951). The reduction of basement membrane and connective tissue ground substance is important. Apparently this change is specific for desquamative gingivitis but the severity may differ.

Omata (p. 799) reports his study of the sensitivity of fusiform organisms to various antibiotics. This study was conducted under laboratory conditions and showed that the fusobacteria were more sensitive to penicillin G and terramycin than to the five other antibiotics tested. The fact that no morphological changes attributable to penicillin were observed in the cultures of these organisms grown in an atmosphere of penicillin is not in accord with the previous finding in the gingival tissue of patients. This suggests that there may be differences in the action of organisms to penicillin in the mouth and the test tube.

Mitchell and Chernausek (p. 802) report the findings of periodontal disease in hamsters. There is great need for a small, economically maintained laboratory animal that may be used for periodontal disease study. Whether the hamster is useful for such investigations is not apparent from this limited study. If debris accumulations are to be used in scoring it should be determined whether the underlying lesions are inflammatory, degenerative, or both. The extent of bone involvement and other factors are also important.

Dunning, Hyde, and Dalton (p. 806) examined over 2,000 psychopathic patients and

found that they had less destruction from caries than had comparable military and industrial personnel. This may be a shock to many who have spoken freely of the direct relationship between mental state and caries, but who never bothered to conduct their study away from the comfortable mental sedative of the investigator's armchair. The only group with increased D.M.F. rate was the depressive psychosis group but the numbers examined were too small to be considered significant.

Morress (p. 815) evaluates the dentition of the Aleut as a criterion of race. The most interesting of his findings is the high percentage of torus mandibularis (35 per cent) of the population showing this anomaly. The method of determining the presence of a torus, i.e., palpation, may account in part for this high figure. It would be interesting to know the same author's findings among a group of Americans.

Kutscher and Chilton (p. 822) were unable to correlate pain threshold, as determined by reaction to radiant heat, with reaction to pain from dental procedures or with the patient's fear of pain or estimate of his pain resistance. It is quite possible that pain reaction to different stimuli may vary. This may be seen clinically in the boxer or football player who does not react adversely to body blows but who reacts violently to insertion of a hypodermic needle, or to the dental bur.

Although the rat has been used for a great many years as an animal for caries studies, and the hamster for several years, few comparisons had been made between the enamel of these animals and the humans. Gilda (p. 828) found the density of rat enamel to resemble that of enamel of human deciduous teeth rather than that of the permanent teeth. The x-ray diffraction pattern of hamster, rat, and human enamel is similar.

The presence of lipids in human dentin has been mentioned in the literature. Leopold, Hess, and Carter (p. 837), from analysis of dentin, have identified cholesterol in dentin. Carter, Leopold, and Hess (p. 840) show that the method of preservation of teeth may affect the dentinal protein recovered for analysis. Although this phenomenon has been overlooked in comparing results of different investigations, it may be of significance.

Investigations of the physical properties of amalgam are quite difficult to conduct because of the variations in the human elements during trituration and packing. Crowell and Phillips (p. 845) utilized clinical methods for preparation of amalgam from alloy in order to study the physical properties in relationship to variations in surface areas. They found that the properties of an alloy cut from different areas of the same mold will vary. They also found variations in the physical properties in relationship to the relative surface area of the alloy. Since amalgam is still widely used as a dental restorative material, there is continued need for more detailed information on its properties. As the physical properties are improved the possibilities of improving the techniques are also advanced.

Henry and Peyton tested the cutting efficiency of dental burs used in a straight handpiece (p. 854). It should be remembered that this study which was conducted on Ivoryene is applicable to dentin rather than to enamel. From a practical standpoint, those who use burs in the mouth will be interested in knowing the tungsten carbide burs cut at essentially the same rate as steel burs but retain their cutting edges longer. However, the carbide burs are well recognized as more brittle and susceptible to breakage. Inverted cone burs were much more efficient than fissure burs in removing material per unit time. Increase in pressure from 500 to 750 grams produced relatively little increase in the amount of material removed. This report should be coordinated with some of the factors of clinical practice in consideration of the production of heat. Because of the effect of heat on the dental pulp it must be given a definite place in the problem.

Various vasoconstrictors have been used in local anesthetic solutions. As pharmacology develops newer agents these should be tested for their efficiency as vasoconstrictors for any undesirable effects. Epstein, Throndson, and Schmitz (p. 870) report the results of a clinical study of arterenol as a vasoconstrictor anesthetic solution on 757 patients. Their findings indicate that arterenol is highly adequate in some regards but that there are other criteria that do not make it the ideal anesthetic. They conclude, however, that arterenol should take its place along with epinephrine and nordefrin as a vasoconstrictor suitable for local anesthetics.

Manly has been investigating masticatory function by different technics. The present study (p. 874) indicates that force of mastication, salivary flow, chewing motion and occlusal balance are of relatively little importance in masticating efficiency in young adults free of periodontal disease. The food platform area and the size of the molars are more important and can be used to predict masticatory efficiency.

The Editor receives numerous "Letters to the Editor." Those which are of interest to many of our readers will be published from time to time with the Editor's Viewpoint.

Dr. McCollum's letter is indicative of the almost passionate zeal with which proponents and opponents of water fluoridation are operating. Of course time and continued *scientific* evaluation will answer the problem.

H. B. G. R.

LETTER TO THE EDITOR

November 9, 1951

Dr. Hamilton B. G. Robinson
Editor of Journal of Dental Research
Columbus, Ohio
Ohio State University

Dear Dr. Robinson:

Recently I have been receiving letters from a number of people who inquire what evidence I have for making the statement attributed to me that "This method (Fluoridation of water supplies for the purpose of reducing susceptibility of human teeth to dental caries) of preventing in some measure the high incidence of dental caries, is in the experimental stage, and so far as I am aware, it has not been found to be effective where it has been tried."

This quotation is said to be from a letter dated August 21, 1950, and signed by me. Since at no time have I ever made such a statement, I was at a loss to know the source of the misinformation. I now find that it is being distributed, along with other quotes unfavorable to fluoridation of water supplies, by the secretary of Citizen Medical Reference Bureau, 1860 Broadway, New York. This information has come to me in letters. I have written the Secretary of that Bureau for information about the source of his quote.

I want the dental profession to be assured that when I have replied to letters asking my views about the value of fluoridation of water, as a preventive measure against dental caries, I have, without exception, stated that the results of studies reported on this subject, have indicated that the measure is safe and has value in caries prevention. Anything you can do to convey this assurance to the profession will be appreciated.

Sincerely,
E. V. McCollum
Professor Emeritus of Biochemistry,
The Johns Hopkins University,
Baltimore, Md.

THE EFFECTS OF AN ACID AND A NEUTRAL SOLUTION OF SODIUM FLUORIDE, ON THE INCIDENCE OF DENTAL CARIES IN YOUNG ADULTS

NORMAN H. RICKLES, D.D.S., M.S., AND HERMANN BECKS, M.D., D.D.S.

From the Division of Dental Medicine, College of Dentistry and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.

THE topical application of sodium fluoride solutions to the teeth of children has been shown by several investigators to be effective in the partial control of dental caries.[4, 7, 8, 11, 12, 13, 14, 15] It is the purpose of this pilot study to determine whether this procedure is also effective in young adults. Furthermore, from some laboratory evidence[19] one might expect that a highly acid solution of sodium fluoride would be more effective clinically in reducing dental caries incidence than a neutral one. Testing this possibility was also a part of this study.

Volker[22] first determined that fluorides decreased enamel and dentin solubility. Muhler and Van Huysen[17, 18] have shown that several fluorides, including NaF, decreased enamel solubility, with tin fluoride being among the most effective. Bibby[2] demonstrated that potassium fluoride was as effective as sodium fluoride in reducing enamel and dentin solubility, but that calcium fluoride had no effect. Buoncore and Bibby[6] found that lead fluoride was even more effective than sodium fluoride in retarding the solubility of powdered enamel. The lower the pH of the solution, the more effective was sodium fluoride in its ability to reduce enamel solubility as shown by Phillips and Muhler.[19] They demonstrated in vitro that the optimum pH of 2.6 was not injurious to the tooth surface, in vitro, during the time of topical application. Phillips and Swartz[20] observed that the hardness of enamel, as measured by resistance to indentation with a sharp instrument, was greatly enhanced by the topical application of various fluoride solutions.

Most clinical investigations indicate that if a 2 per cent unbuffered aqueous solution of sodium fluoride was applied* to the teeth of children up to the age of 15 years, 4 times, either once a week or semi-weekly, dental caries could be controlled to the extent of approximately 40 per cent.[7, 8, 11, 12, 13, 14, 15] According to a study on young adults by Klinkenberg and Bibby,[10] 44.5 per cent less new DMF carious surfaces occurred after 1 year in teeth treated with 1 per cent NaF than in the control teeth. Galagan and Knutson[8] demonstrated that a 1 per cent solution of sodium fluoride was equally effective as a 2 per cent solution. Bibby[4] found that even a 0.1 per cent solution significantly reduced dental caries incidence. Clinical trials using a sodium fluoride mouthwash with

Aided by grants from the California State Dental Association, the American Foundation for Dental Science, Army Contract W49-007-MD-470 and the Regents of the University of California.

Received for publication, June 6, 1951.

*According to the method recommended by the U.S. Public Health Service.[13]

Table I

Effect of a Neutral 2% Sodium Fluoride Solution on Individual Dental Caries Incidence

Number of Individuals	Age	Dental Caries Present at Initial Examination										New Dental Caries Present at Final Examination									
		Treated Halves					Untreated Halves					Treated Halves					Untreated Halves				
		BCI*	DMF		Inter-proximal	Secondary	BCI	DMF		Inter-proximal	Secondary	BCI	DMF		Inter-proximal	Secondary	BCI	DMF		Inter-proximal	Secondary
			Surfaces	Teeth				Surfaces	Teeth				Surfaces	Teeth				Surfaces	Teeth		
1	22	36(10)**	33(8)	13	14(11)	3	26(6)	24(5)	11	6(2)	1	2	2	0	0	0	4	4	1	3	0
2	23	18(6)	16(5)	7	8(4)	0	17(6)	15(5)	6	6(4)	0	0	0	0	0	0	2	2	2	2	0
3	23	3(2)	3(2)	3	3(2)	0	7(6)	7(6)	5	6(5)	0	7	7	4	3	0	7	6	0	1	0
4	24	22(6)	20(5)	10	13(4)	0	24(3)	22(3)	11	10(2)	0	1	1	1	0	0	2	2	1	0	0
5	24	29(17)	27(15)	11	16(14)	1	30(14)	28(12)	10	11(9)	0	1	1	0	0	0	0	0	0	0	0
6	24	34(8)	31(7)	11	15(5)	4	30(8)	28(7)	11	9(3)	1	1	1	0	0	0	1	1	0	0	0
7	24	16(7)	14(6)	7	6(4)	0	18(7)	16(6)	10	8(5)	0	0	0	0	0	0	2	2	0	1	1
8	25	31(10)	29(9)	12	20(8)	0	27(17)	25(16)	13	17(15)	1	1	1	0	0	0	3	3	1	1	0
9	25	28(19)	24(16)	10	13(12)	0	24(19)	22(18)	10	13(11)	0	1	1	0	1	0	5	6	1	3	0
10	25	34(13)	31(12)	11	13(10)	0	29(10)	27(9)	11	15(9)	0	0	0	0	0	0	2	2	2	2	0
11	25	38(14)	34(13)	13	14(6)	2	37(20)	34(19)	12	14(8)	1	4	4	0	1	0	3	3	0	1	1
12	26	23(11)	21(10)	10	4(3)	1	31(14)	29(13)	12	7(6)	2	1	1	0	1	0	2	2	0	1	0
13	26	38(10)	34(9)	13	23(9)	4	39(6)	36(5)	13	23(5)	0	0	0	0	0	0	0	0	0	0	1
14	26	4(4)	4(4)	2	2(2)	0	0(0)	0(0)	0	0(0)	0	5	5	4	1	1	6	5	4	3	0
15	27	25(8)	21(7)	9	7(3)	3	28(20)	26(19)	8	15(9)	1	9	9	2	3	2	10	9	3	1	3
16	27	19(17)	17(16)	11	9(8)	1	25(18)	21(17)	10	11(9)	2	0	0	0	0	0	4	4	1	1	0
17	27	28(9)	26(8)	10	12(7)	0	26(11)	24(10)	11	12(7)	0	3	3	0	1	1	3	3	0	1	0
18	27	21(16)	19(15)	9	7(6)	1	16(11)	14(10)	8	4(4)	0	3	3	0	3	0	6	5	0	2	0
19	28	31(4)	29(3)	13	14(3)	1	28(11)	26(10)	11	12(6)	2	0	0	0	0	0	2	2	0	1	1
20	29	26(14)	23(13)	9	13(9)	0	24(7)	22(6)	8	9(3)	0	1	1	0	1	1	0	0	0	0	1
21	29	17(7)	16(6)	8	4(4)	2	20(14)	18(13)	9	8(6)	0	0	0	0	0	1	2	2	0	2	0
22	30	36(11)	32(10)	12	19(8)	6	39(16)	36(14)	14	17(11)	2	1	1	0	0	0	1	0	1	1	1
23	31	21(3)	19(3)	8	11(2)	1	24(6)	22(5)	11	12(4)	0	3	3	2	1	0	3	3	1	1	0
24	33	9(8)	7(4)	5	2(0)	0	10(4)	8(3)	6	0(0)	0	0	0	0	0	0	2	2	1	0	0
25	34	22(15)	20(14)	8	10(7)	0	19(10)	17(9)	7	8(5)	0	1	1	0	0	0	2	2	0	1	0
Total		593 (264)	541 (240)	238	274 (163)	30	603 (266)	554 (245)	241	261 (148)	13	48	48	13	16	5	80	76	16	32	9

*Bodecker Caries Index (6)

**Parentheses indicate the number of open carious lesions; e.g. - of the total of 36 carious counts, 10 are due to open lesions present at the initial examination.

an acid reaction[3, 21] showed no reduction of dental caries; neither did an acidulated sodium fluoride solution applied once to the teeth of young adults.[1] Knutson, Armstrong and Feldman[14] using 2, 4 and 6 applications of a 2 per cent sodium fluoride solution without prior prophylaxis on 1,458 children produced 9.3, 20.1 and 21.3 per cent reductions, respectively. This was half as effective as when prophylaxis was given by Galagan and Knutson in a later study.[7]

METHOD AND MATERIAL

Forty-seven freshman and sophomore dental students between the ages of 22 and 34 years (average 27 years) were given dental examinations in 1947, including anterior and posterior bite-wing roentgenograms. All examinations and roentgenographic interpretations were made by the same person. Missing teeth, fillings, and carious areas were recorded carefully. Whenever a tooth was missing an attempt was made to determine the reason for its absence. The carious areas (past and present) were recorded according to the modified Boedecker caries index.[5] Twenty-five students (Group 1) received 4 applications of a 2 per cent NaF solution* at a pH of 7 at 3-monthly intervals to the teeth on one side of their mouth, while the teeth on the other side were untreated and served as controls. The same solution, however, adjusted to a pH of 3.5 by phthalate-HCl buffer, was applied to the other 22 students (Group 2).

The clinical technique employed was as follows: (1) A thorough prophylaxis was given and the patient's mouth was rinsed with water. (2) The teeth on one side of the mouth were isolated with cotton rolls, dried and coated with a cavity varnish.** (3) The cotton rolls were then removed and the opposite side was isolated. (4) The surfaces of the teeth to be treated were dried and the sodium fluoride solution was applied with cotton pellets. (5) The teeth were kept wet for four minutes and allowed to dry for five minutes, after which the patient's mouth was rinsed with water.

At the outset of the experiment, a 7-day food intake record was kept by each student for an evaluation of their general food habits, with particular reference to the fermentable carbohydrate ingestion. After two years, roentgenographic and dental examinations were repeated and compared with the original records by the same investigator who made the initial examinations. In order to rule out the possibility of personal bias, he never knew to which group any test subject belonged. New dental caries which had developed during the two-year period was recorded again according to the modified Boedecker Caries Index (BCI).[5]

RESULTS AND DISCUSSION

Because of the small number of test subjects, the results are only preliminary in nature. A more comprehensive follow-up study is now in progress.

*Sodium Fluoride 10 Gm.
Sodium lauryl sulfate 1.5 Gm.
Distilled water q.s. 500 c.c.

**Repelac.

Table II

Effect of an Acid 2% Sodium Fluoride Solution on Individual Dental Caries Incidence

Number of Individuals	Age	Dental Caries Present at Initial Examination										Dental Caries Present at Final Examination									
		Treated Halves					Untreated Halves					Treated Halves					Untreated Halves				
		SCI*	DMF Surfaces	DMF Teeth	Interproximal	Secondary	SCI	DMF Surfaces	DMF Teeth	Interproximal	Secondary	SCI	DMF Surfaces	DMF Teeth	Interproximal	Secondary	SCI	DMF Surfaces	DMF Teeth	Interproximal	Secondary
1	21	20(15)**	18(14)	10	6(6)	0	18(12)	17(11)	10	4(4)	0	6	5	2	4	0	5	5	1	1	0
2	22	21(4)	19(3)	9	6(3)	0	32(12)	30(11)	11	9(5)	0	1	1	0	0	0	1	1	0	1	1
3	23	27(8)	25(7)	10	11(6)	0	29(8)	27(7)	11	18(6)	1	1	1	1	0	0	2	2	0	0	1
4	23	17(0)	15(0)	7	7(0)	0	29(11)	27(10)	10	12(6)	0	0	0	0	0	0	1	1	0	0	0
5	23	21(9)	19(8)	7	10(7)	1	25(10)	23(9)	9	9(7)	0	7	6	1	2	0	5	4	1	2	0
6	24	28(11)	24(10)	10	15(8)	3	20(11)	18(10)	7	10(6)	0	5	5	1	2	1	1	1	0	0	1
7	24	29(24)	27(23)	11	14(12)	1	32(24)	30(23)	12	18(15)	0	3	2	0	1	0	3	2	0	0	0
8	25	18(7)	16(6)	8	7(5)	0	17(8)	16(8)	7	7(3)	0	2	2	0	0	1	5	4	1	2	0
9	26	22(13)	20(12)	8	10(9)	1	26(17)	23(16)	8	12(12)	0	4	4	0	1	1	5	4	0	1	0
10	26	13(9)	12(8)	7	6(6)	0	9(6)	8(6)	5	5(5)	0	4	4	0	2	0	3	3	0	0	0
11	27	27(8)	25(7)	10	11(5)	1	24(9)	22(8)	8	9(4)	1	0	0	0	0	0	1	1	0	0	0
12	27	29(18)	27(18)	11	18(18)	1	26(19)	24(18)	11	14(11)	0	6	6	2	1	0	6	5	1	0	1
13	27	19(9)	17(8)	9	10(8)	0	17(9)	16(7)	8	8(6)	0	2	2	0	0	0	2	2	1	1	0
14	28	22(8)	20(5)	8	10(4)	0	23(9)	21(8)	11	11(6)	0	5	5	1	0	2	3	2	0	0	0
15	28	21(5)	19(5)	9	7(0)	0	19(5)	17(5)	8	6(0)	0	4	4	0	1	0	5	4	1	3	2
16	28	20(11)	18(10)	13	11(9)	1	24(17)	22(16)	13	16(15)	0	4	4	1	2	0	3	3	1	2	0
17	29	16(5)	14(5)	9	7(3)	1	17(4)	16(4)	6	8(3)	0	2	2	1	0	0	1	1	1	1	0
18	29	17(11)	15(10)	8	8(8)	0	27(20)	25(19)	11	12(12)	0	6	5	1	1	1	3	3	0	0	0
19	29	39(8)	37(8)	10	6(4)	0	38(7)	33(6)	11	11(5)	1	3	3	1	1	1	4	3	1	1	0
20	29	8(6)	6(5)	4	0(0)	0	15(13)	12(12)	7	4(4)	0	7	6	4	4	0	4	3	4	1	0
21	30	14(3)	12(3)	7	3(2)	0	12(6)	11(6)	8	7(6)	1	2	2	0	1	0	8	7	2	2	0
22	31	26(9)	24(8)	11	10(9)	0	31(8)	29(7)	12	18(8)	4	2	2	0	1	0	3	3	1	0	0
Total		469 (197)	428 (181)	197	196 (124)	10	806 (239)	667 (223)	206	221 (145)	8	75	71	16	24	7	72	62	16	10	6

*Knutson Caries Index (S)

**Parentheses indicate the number of present open carious lesions; e.g. - of the total of 20 carious counts, including both past and present caries, 15 of these counts are due to open lesions present at the initial examination.

Group I. Individuals treated with a neutral NaF solution (Table I).—Past dental caries in each individual and in aggregate approached bilateral symmetricity. Therefore, it was assumed that for future dental caries, the same degree of bilateral symmetricity could be expected unless some of the teeth were treated with an agent interfering with the dental caries increment. At the completion of the two-year observation period, in 16 cases more dental caries was observed in the untreated than in the treated halves. Six individuals developed an equal amount of caries on both sides, while only 3 incurred more in the treated than in the untreated teeth. Forty-eight new lesions (as measured by the BCI) occurred on all the treated halves as compared with 80 on the untreated halves. Using the DMF surfaces method of dental caries evaluation, the figures were 48 and 76, respectively; if employing the DMF teeth method, 13 additional treated and 16 more untreated teeth were involved. Considering interproximal dental caries only, 16 new lesions appeared in the treated areas while 32 developed in the untreated. Five areas of secondary dental caries appeared in the treated against 9 in the untreated teeth. Thus a definite inhibition of dental caries by the neutral 2 per cent NaF solution was attained.

Group II. Individuals treated with an acid sodium fluoride solution (Table II).—In this group, as in Group I, approximative bilateral symmetricity of past dental caries was found. During the 2-year observation period 9 individuals developed more lesions in the treated than in the control teeth, 3 showed an equal increment of dental caries bilaterally, while 10 demonstrated a greater number in the control than in the treated halves.

Seventy-five new carious surfaces (BCI) developed in all treated areas while 72 appeared in the untreated ones. Comparing this with the DMF surfaces measurement, the numbers were 71 and 62, respectively. Sixteen new DMF teeth were involved on both halves. Interproximally, 24 lesions developed on the treated sides and 18 on the untreated. New secondary dental caries occurred 7 times in the treated and 6 in the untreated teeth. Therefore, the acid NaF solution does not appear to have had an inhibitory effect on the progress of dental caries.

Table III represents a summary of the findings. Percentages of dental caries reduction were calculated as follows: $\frac{U - T}{U} \times 100$; U represents the number of new lesions in the untreated halves and T the number of new lesions in the treated halves. Using the BCI, DMF surfaces, and DMF teeth methods for Group I, dental caries was reduced to the extent of 40.0, 36.8, and 18.7 per cent. Interproximal dental caries was reduced 50.0 per cent and secondary lesions, 44.4 per cent.

In comparing the final with the initial examinations, the percentage of increase in open lesions in the experimental teeth (BCI) was found to be 48/264 or 18 per cent, and in the controls, 80/266 or 30 per cent. The difference between these two percentages proved to be statistically significant[9]; i.e., the chances are that on the average 99.9 out of 100 young adults treated with a neutral 2 per cent sodium fluoride solution will develop less dental caries than

Table III

Summary of Tables I & II

Group	Dental Caries Index	Initial Examination: Total Dental Caries Filled & Open Lesions: Treated Teeth	Initial Examination: Total Dental Caries Filled & Open Lesions: Untreated Teeth	Initial Examination: Total Open Lesions: Treated Teeth	Initial Examination: Total Open Lesions: Untreated Teeth	Final Examination: Total New Dental Caries: Treated Teeth	Final Examination: Total New Dental Caries: Untreated Teeth	Final Examination: Total New Dental Caries: % Diff.	Initial Examination: Interproximal Dental Caries Filled & Open Lesions: Treated Teeth	Initial Examination: Interproximal Dental Caries Filled & Open Lesions: Untreated Teeth	Initial Examination: Interproximal Open Lesions: Treated Teeth	Initial Examination: Interproximal Open Lesions: Untreated Teeth	Final Examination: New Interprox. Dental Caries: Treated Teeth	Final Examination: New Interprox. Dental Caries: Untreated Teeth	Final Examination: New Interprox. Dental Caries: % Diff.	Initial Examination: Secondary Dental Caries: Treated Teeth	Initial Examination: Secondary Dental Caries: Untreated Teeth	Final Examination: New Secondary Dental Caries: Treated Teeth	Final Examination: New Secondary Dental Caries: Untreated Teeth	Final Examination: New Secondary Dental Caries: % Diff.
I	Surfaces (Boedecker)	583	603	264	266	48	80	40.0	274	251	163	148	16	32	50.0	30	13	8	9	44.4
(Neutral NaF)	Surfaces DMF	541	554	240	243	48	76	36.8	274	251	163	148	16	32	50.0					
	Teeth DMF	238	241			13	16	18.7												
II	Surfaces (Boedecker)	469	504	197	239	75	72	-4.2	196	221	124	145	24	18	-33.3	10	8	7	6	-15.7
(Acid NaF)	Surfaces DMF	428	467	181	223	71	62	-14.5	196	221	124	145	24	18	-33.3					
	Teeth DMF	197	205			16	16	0												

those who were not so treated. Similarly, significant differences in percentages were found for interproximal and secondary dental caries. However, when DMF teeth were used in measuring dental caries, the differences were not significant, although a trend toward dental caries reduction existed.

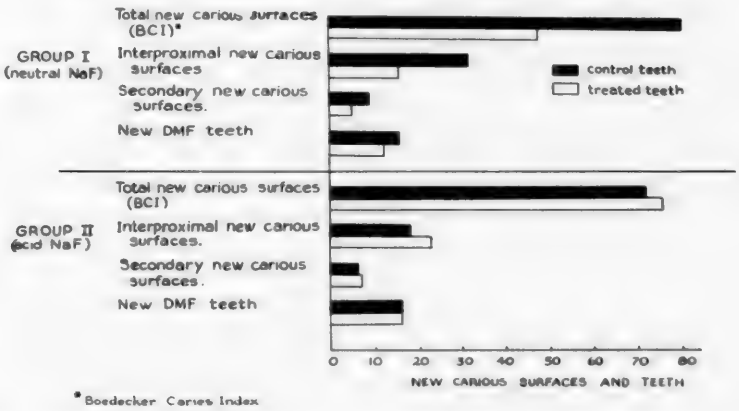
When an acid NaF solution was used, no significant difference in dental caries reduction between treated and untreated areas was found. Actually, more dental caries occurred in the treated than in the control teeth, but these differences proved to be statistically insignificant.

The increases in dental caries in Groups I and II are demonstrated graphically in Table IV.

It can be seen immediately that the effects of the two solutions were different. While the neutral 2 per cent solution of NaF definitely reduced the dental caries incidence of young adults over a 2-year period, the same solution at an acid pH had no such effect. The negative clinical results in the latter group do not confirm in vitro experiments. On the other hand, the effect of the neutral solution is consistent with the findings of other investigators in similar clinical studies.

TABLE IV

COMPARISON OF DENTAL CARIES REDUCTION IN GROUPS I AND II



*Boedecker Caries Index

The value of the sodium lauryl sulfate is difficult to assess since no control group was used. This surface-active agent, which does not interfere with the action of the fluoride ion, should actually wet more areas than would be possible with a high surface tension aqueous solution. However, in comparing the results of this study with those in which no wetting agent was used, it appears that the sodium lauryl sulfate was of no particular aid.

In computing percentage of dental caries reduction, the three indices gave varying results. The greatest percentage of reduction was obtained when the BCI was used. The DMF surfaces method resulted in a somewhat smaller percentage, although it was of the same order of magnitude. When DMF teeth were used the percentage of reduction was only about one-half of that obtained

with DMF surfaces. Whether these differences are an indication of the sensitivity of the three methods for measuring dental caries will be determined in the follow-up study.

The 7-day individual food intake records indicated that most of the subjects had a near-optimal diet, however, with the addition of large quantities of fermentable carboyhdrates. In evaluating the possible dental caries-reducing effect of the fluoride solution and the stimulating effects of the carbohydrates, it is evident that the latter of course exerted their action bilaterally, while the solution was applied only to one side of the mouth.

SUMMARY AND CONCLUSIONS

The effects of topically applied neutral and acid (pH 3.5) NaF solutions on the incidence of dental caries in young adults was tested. Sodium lauryl sulfate was incorporated into each solution as a wetting agent.

A 2 per cent solution of NaF, at a pH of 7, was capable of significantly reducing the total, interproximal and secondary dental caries incidence.

A 2 per cent solution of NaF, at a pH of 3.5, did not reduce dental caries incidence.

REFERENCES

1. Arnold, F. A., Jr., Dean, H. T., and Singleton, D. E.: The Effect on Caries Incidence of a Single Topical Application of a Fluoride Solution to the Teeth of Young Adult Males of a Military Population, *J. D. Res.* **23**: 155, 1944.
2. Bibby, B. G.: Effectiveness of Various Fluoride Preparations in Reducing Tooth Solubility, *J. D. Res.* **23**: 202, 1944.
3. Bibby, B. G., Zander, H. A., McKelleget, M., and Labunsky, B.: Preliminary Reports on the Effect on Dental Caries of the Use of Sodium Fluoride in a Prophylactic Cleaning Mixture and in a Mouthwash, *J. D. Res.* **25**: 207, 1946.
4. Bibby, B. G.: The Use of Fluorine in the Prevention of Dental Caries II, *J. A. D. A.* **31**: 317, 1944.
5. Boedecker, C. F.: The Modified Dental Caries Index, *J. A. D. A.* **26**: 1453, 1939.
6. Buoncore, M. G., and Bibby, B. G.: The Effects of Various Ions on Enamel Solubility, *J. D. Res.* **24**: 103, 1945.
7. Galagan, D. J., and Knutson, J. W.: Effect of Topically Applied Fluorides on Dental Caries Experience. V. Report of Findings with 2, 4, and 6 Applications of Sodium Fluoride and of Lead Fluoride, *U. S. Public Health Rep.* **62**: 1477, 1947.
8. Galagan, D. J., and Knutson, J. W.: Effect of Topically Applied Fluoride on Dental Caries Experience. VI. Experiments with Sodium Fluoride and Calcium Chloride . . . . Widely Spaced Applications . . . . Use of Different Solution Concentrations, *U. S. Public Health Rep.* **63**: 1215, 1948.
9. Garrett, H. E.: Statistics in Psychology and Education, ed. 3, New York, London, Toronto, 1947, Longmans, Green, and Co., Ch. 9, p. 219.
10. Klinkenberg, E., and Bibby, B. G.: The Effect of Topical Applications of Fluorides on Dental Caries in Young Adults, *J. D. Res.* **29**: 4, 1950.
11. Knutson, J. W., and Armstrong, W. D.: The Effect of Topically Applied Sodium Fluoride on Dental Caries Experience. I. Report of Findings for First Study Year, *U. S. Public Health Rep.* **58**: 1701, 1943.
12. Knutson, J. W., and Armstrong, W. D.: The Effect of Topically Applied Sodium Fluoride on Dental Caries Experience. II. Report of Findings for Second Study Year, *U. S. Public Health Rep.* **60**: 1085, 1945.
13. Knutson, J. W., and Armstrong, W. D.: The Effect of Topically Applied Sodium Fluoride on Dental Caries Experience. III. Report of Findings for Third Study Year, *U. S. Public Health Rep.* **61**: 1683, 1946.
14. Knutson, J. W., Armstrong, W. D., and Feldman, F. M.: The Effect of Topically Applied Sodium Fluoride on Dental Caries Experience. IV. Report of Findings With 2, 4, and 6 Applications, *U. S. Public Health Rep.* **62**: 425, 1947.
15. Knutson, J. W.: An Evaluation of the Effectiveness as a Caries Control Measure of the Topical Application of Solutions of Fluorides, *J. D. Res.* **27**: 340, 1948.

16. Knutson, J. W.: Sodium Fluoride Solutions: Technique for Application to the Teeth, *J. A. D. A.* **36**: 37, 1948.
17. Muhler, J. C., and Van Huysen, G.: Solubility of Enamel Protected by Sodium Fluoride and Other Compounds, *J. D. Res.* **26**: 119, 1947.
18. Muhler, J. C., and Van Huysen, G.: Sodium Fluoride Reagents Concentration and Powdered Enamel Solubility, *J. D. Res.* **27**: 142, 1948.
19. Phillips, R. W., and Muhler, J. C.: Solubility of Enamel as Effected by Fluorides of Varying pH, *J. D. Res.* **26**: 109, 1947.
20. Phillips, R. W., and Swartz, M. L.: Effect of Fluorides on Hardness of Tooth Enamel, *J. A. D. A.* **37**: 1, 1948.
21. Roberts, J. F., Bibby, B. G., and Wallach, W. D.: Effect of an Acidulated Fluoride Mouthwash on Dental Caries, *J. D. Res.* **27**: 497, 1948.
22. Volker, J. M.: Effect of Fluorine on Solubility of Enamel and Dentin, *Proc. Soc. Exper. Biol. and Med.* **42**: 725, 1939.

BACTERIOLOGIC STUDIES OF THE ADVANCING DENTINAL LESION

GEORGE W. BURNETT,* D.D.S., PH.D., AND HENRY W. SCHERP, PH.D.

The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

IF CERTAIN microorganisms are found regularly in close proximity to the advancing front of dentinal caries they should be seriously considered as etiologically significant. Perhaps more importantly, a study of the reactions which these organisms are able to effect would be indicative of the various processes which occur in the initial stages of the disease in dentin. Miller,[1] in one of the first histological investigations of dentinal caries, found no bacteria in the softened dentin immediately adjacent to the intact, unharmed portions. Goadby,[2] on the other hand, cultured the material from deep dentinal caries and found that *Streptococcus brevis, Bacillus necrodentalis,* and *Staphylococcus albus* were present. Some years later Gies,[3, 4] Gies, Hull, and Mullikin,[5] and Gies and Kligler[6, 7, 8] found that *Bacillus acidophilus* (Moro) and a filamentous form designated as *Cladothrix placoides* could be isolated regularly from deep dentinal caries. Clarke,[9] however, was unable to find *B. acidophilus* in deep carious dentin, except in very shallow cavities. In 72 per cent of the cases he did find a small, pleomorphic bacterium which he called *Streptococcus mutans.* Bunting and Palmerlee,[10] and Bunting, Nickerson and Hard[11] found a similar organism which they called *B. acidophilus.* They believed that Clarke's *Str.. mutans* was *B. acidophilus* grown under alkaline conditions and felt there was no justification for its classification as a streptococcus. MacLean[12] isolated only *B. acidophilus* and *Str. mutans* from deep dentinal caries. He was also of the opinion that *Str. mutans* was identical with *B. acidophilus* as described by Bunting and co-workers.[10, 11] Canby and Bernier[13] studied the bacterial flora of shallow and deep portions of dentinal caries. They found that no bacteria could be cultivated from the deeper layers of carious dentin in 36 per cent of the cases. *Lactobacillus acidophilus* was found in over 90 per cent of all cases from which bacteria could be cultivated. Staphylococci and streptococci were also present. In spite of the presence of cultivable bacteria in most deep dentinal caries, Canby and Bernier were of the opinion that the effective organisms were somewhat back from the area in which decalcification was actually occurring. Based on recent histological studies, Sognnaes and Wislocki[14] have concluded that decalcification proceeds ahead of an actual invasion by spheroidal microorganisms. On the other hand, other investigators[15] have published photographs of various bacteria invading intact dentin in the carious process.

The data presented in this paper were taken from a thesis submitted in June 1950 by George W. Burnett to the University of Rochester in partial fulfillment of the requirement for the degree, Doctor of Philosophy. The study was supported by a Contract (W49-007 MD 394) between the University of Rochester and the Army Medical Service Research and Development Board, Office of the Surgeon General, Department of the Army.

Received for publication, June 11, 1951.

*Present address: Dental Division, Army Medical Service Graduate School, Army Medical Center, Washington 12, D. C.; Major, DC, U. S. Army.

The evidence obtained by the various investigators indicates, therefore, that microorganisms do invade the area in dentin where it is presumed that the carious process is actually occurring. In order to facilitate invasion, it seems likely that the acids or enzymes produced by these microorganisms must act upon the dentin ahead of the actual invasion. The earlier work on the advancing carious front deep in dentinal caries was largely bacteriological, but it was either incomplete, inconsistent, or it described microorganisms not readily defined by modern bacteriology. Later investigations have been largely histological and have defined the pathological picture of dental caries, but cannot establish the nature of the microbial flora. For this reason it was felt that a renewed bacteriological study of the advancing carious front deep in dentinal caries would yield additional information as to the nature of the processes occurring at this location.

METHODS

Teeth with deep dentinal caries were obtained immediately after extraction and were placed in separate sterile Petri dishes. Cavities with minimal enamel openings were selected for study, for the reason that they would have been least exposed to saliva. The enamel around the cavities was broken down and the cavities were exposed. The decalcified, leathery dentin was enucleated from the cavity by the use of successive sterile dentin excavators. Especial precautions were observed to avoid touching and contaminating the advancing carious front at the bottom of the cavity. After enucleating the decalcified dentin and exposing the junction between decalcified and intact dentin, the junction was thoroughly scraped with a sterile No. 66 spoon excavator. In early experiments, 10 mg. samples of the scrapings were ground in a sterile mortar with 5 ml. of Douglas' broth. In later tests, some of the samples were homogenized in Douglas' broth with the aid of sterile glass beads. Both methods yielded comparable results. Apparently the incomplete disintegration of the samples by the use of glass beads was offset by the killing of a certain number of bacteria when the samples were ground in a mortar. After homogenizing the specimens, decimal dilutions of the suspensions were made, and 0.1 ml. samples were cultured anaerobically and aerobically at 37.5°C. for 4 days, on tomato juice agar (pH 5.0), 40 per cent milk agar plates, egg plates coagulated at 80°C., and finally on blood agar plates. Colony counts were then made, using a wide field microscope at 9× magnification. Additional details of the cultural methods and a comparison of the distribution of proteolytic and aciduric bacteria in other levels of the carious lesions have been previously reported.[16]

In addition to the determination of the number of cultivable bacteria, the various colonial types were differentiated. A number of strains, representative of the several types, were isolated in pure culture and subjected to a detailed biochemical and cultural study for the purpose of classification. In addition to the usual tests for biochemical activity, the gram-positive, nonsporulating rods, i.e., lactobacilli, were subjected to metabolism balance studies to determine

TABLE I

SUMMARY OF THE BACTERIAL COUNTS OF MATERIAL OBTAINED FROM DEEP DENTINAL CARIES*

	LACTOBACILLI		OTHER ACIDURIC ORGANISMS		OVOLYTIC BACTERIA		CASEINOLYTIC BACTERIA		TOTAL MICRO-ORGANISMS	
	A	AN	A	AN	A	AN	A	AN	A	AN
Numerical mean	0	53,000	17,000	17,000	Actual count‡ 5,000 40,000	0	59,000	439,000	636,000	1,055,000
Standard deviation	0	26,400	18,400	16,900	†	0	23,800	255,000	296,000	647,000
Numerical range	0	30,000-80,000	0-60,000 (5 neg.)	0-35,000 (2 neg.)		0	25,000-100,000	200,000-800,000	200,000-1,200,000	300,000-2,500,000
Mean %	0	5.4	3.3	1.8	0.033	0	8.7	52.0		
Range %	0	3.0-10.0	0-11.3	0-5.0		0	3.6-12.5	22.0-83.0		

*Colonies per gram.
†Meaningless because of the number of negative cultures.
‡Only two cases were positive.
Key: A = Aerobic Cultivation (all 15 cases).
AN = Anaerobic Cultivation (9 cases).

whether they were homo- or heterofermentative when utilizing glucose. The concentrations of glucose and lactic acid in cultures in trypticase soy broth were determined in Somogyi filtrates[17] by the methods of Schales and Schales,[18] and Barker and Summerson,[19] respectively. The gram-positive cocci were subjected to the usual biochemical tests, and to tests to ascertain their growth patterns. In addition, the cocci were tested for catalase production according to the method of Isaacs and Scouller.[20] While the filamentous organisms were subjected to biochemical and growth tests, certain modifications were made in these procedures, the most important being that of testing the fermentability of sugars in thioglycollate broth (glucose-free).

RESULTS

A summary is shown in Table I of the results of the colony counts of material cultured from the deeper layers of 15 dentinal carious lesions. The total numbers of microorganisms varied somewhat when the samples were incubated aerobically and anaerobically. Aerobically, the numbers of bacteria ranged up to 1,200,000 per gram of dentin sample, with a mean value of 636,000. Anaerobically, the total cultivable bacterial population was as high as 2,500,000 per gram, with a mean value of 1,055,000. A statistical analysis of these figures showed that the differences between the numbers of bacteria obtained by aerobic and anaerobic cultivation were not significant.

Under aerobic growth conditions gram-positive cocci were the only bacterial types isolated regularly from the 15 lesions studied. Ovolytic types were found in only two cases, but caseinolytic forms were present regularly, accounting for 8.7 per cent of the total, on the average. Only 3.3 per cent of the cocci were aciduric; none were found in 5 cases. Because aciduric organisms were deemed of greater immediate interest for the purpose of the present investigation, 8 strains of aciduric cocci were isolated from 8 separate lesions for further study.

When the carious material was cultured anaerobically, as was done in 9 of 15 cases, a somewhat different distribution of bacteria was found. Gram-positive cocci still constituted over 90 per cent of the total. The proportion of aciduric cocci decreased slightly (average = 1.8 per cent) and ovolytic organisms were not found. The proportion of caseinolytic cocci, however, rose sixfold to an average value of 52 per cent. In addition, gram-positive, nonsporulating bacilli, subsequently shown to be lactobacilli, were present in every case on tomato juice agar. These lactobacilli constituted from 3 to 10 per cent of the total, averaging 5.4 per cent. Ten strains were isolated from 9 separate lesions for further study. As the investigation progressed, it was ascertained that a gram-positive, pleomorphic filamentous microorganism also was present upon the tomato juice agar at pH 5.0 when incubated anaerobically, although it did not form well-defined colonies. This organism was obtained regularly from deep dentinal caries, but it could never be induced to form discrete colonies on primary culture on tomato juice agar. Growth usually occurred as a diffuse hazy

film which could be distinguished easily from the normal surface of the agar. By scraping the surface of the medium between the colonies of bacilli and cocci, the organism could be recovered and subcultured in thioglycollate broth in which it grew slowly at first. After subculturing for some time, it became less fastidious, growing more rapidly in thioglycollate broth and forming colonies on tomato juice agar at pH 6.0. Ten strains of this organism were isolated from 9 separate lesions for a detailed study.

Our investigation showed, therefore, that only three types of bacteria could be cultivated from 15 cases of the advancing carious lesion in dentin. The cocci were the most abundant and constituted all of the cultivable forms other than the lactobacilli and the filamentous types. Only two strains of the cocci were obtained which were ovolytic, though many strains were caseinolytic. These

TABLE II

THE CHARACTERISTICS OF THE GRAM-POSITIVE COCCI FROM DENTINAL CARIES

	STRAINS							
	1	2	3	4	5	6	7	8
Final pH in 1% glucose broth	4.8	5.1	4.7	4.9	5.0	4.9	5.2	5.1
Final pH in 1% glucose broth plus dentin	5.6	5.7	5.6	5.5	5.7	5.6	5.7	5.6
Hemolysis of blood	*	*	*	*	*	*	*	*
Liquefaction of gelatin	*	*	*	*	*	*	*	*
Growth in 0.02% sodium azide broth	†	†	†	†	†	†	†	†
Growth in methylene blue milk {0.1%	†	†	†	†	†	†	†	†
Growth in methylene blue milk {0.3%	†	†	†	†	†	†	†	†
Growth in broth containing 6.5% NaCl	†	†	†	†	†	†	†	†
Growth in bile agar {10%	†	†	†	†	†	†	†	†
Growth in bile agar {40%	†	†	†	†	†	†	†	†
Growth in broth adjusted to pH 9.5-9.6	†	†	†	†	†	†	†	†
Growth at 10° C.	†	‡	†	†	‡	†	‡	†
Growth at 45° C.	†	†	†	†	†	†	†	†
Litmus milk reduced before clotting	†	†	†	†	†	†	†	†
Catalase production	np	p	np	np	q	np	q	np

Key: *No. growth.
†Growth.
‡Growth questionable.
p Production.
np Nonproduction.
q Production questionable.

ovolytic cocci were not used in the subsequent study because they were not consistently present. The cocci of all types were more abundant when cultured anaerobically. Anaerobic lactobacilli were present consistently, though they were not as abundant as the cocci (5 per cent as compared to over 90 per cent). The proportion of filamentous microorganisms could not be determined for they did not form distinct colonies upon primary isolation. They were, however, far from rare.

The cultural characteristics of 8 strains of the aciduric cocci, cultured from 8 separate lesions, are found in Table II. They were facultatively anaerobic upon primary isolation. They produced a typical colonial form resembling *Staph. albus*. They were strongly gram-positive and seldom formed chains of more than from 3 to 5 cells. In Douglas' broth containing one per cent glucose,

they produced a terminal pH of 4.7 to 5.2. In the same medium supplemented by dentin (20 to 35 mg. dentin in 5 ml. of medium) they produced a higher terminal pH, i.e., 5.5 to 5.7. They did not hemolyze rabbit blood or liquefy gelatin. They grew in 0.02 per cent sodium azide broth, in 0.1 and 0.3 per cent methylene blue milk, in 6.5 per cent sodium chloride, on 10 and 40 per cent bile agar, in Douglas' broth adjusted to pH 9.5 to 9.6, and at 10° and 45°C. All strains reduced litmus milk before producing a clot. These characteristics indicated that the gram-positive cocci were enterococci, chiefly because they were able to grow when subjected to certain rather stringent environmental conditions.[21] Isaacs and Scouller,[20] however, differentiated many such organisms into micrococci and enterococci on the basis that the former produced catalase, whereas the latter did not do so. When tested by the method of these authors, our strains 1, 3, 4, 6, and 8 were devoid of the enzyme but strains 2, 5, and 7 were shown to produce it. The latter three strains, therefore, had to be classed as micrococci in spite of the predominant characteristics in which they resembled the enterococci.

TABLE III

CHARACTERISTICS OF THE GRAM-POSITIVE, NONSPORULATING BACILLI FROM THE CARIOUS LESION

	STRAINS									
	1	2	3	4	5	6	7	8	9	10
Anaerobic on primary culture	†	†	†	†	†	†	†	†	†	†
Survival at pH 3.0 for 48 hrs.	†	†	†	†	†	†	†	†	†	†
Growth in lactic acid broth at pH 5.0	†	†	†	†	†	†	†	†	†	†
Growth on tomato juice agar at pH 5.0 (lactic acid)	†	†	†	†	†	†	†	†	†	†
Lactic acid from glucose**	82	87	98	93	86	85	94	84	95	90
Liquefaction of gelatin	*	*	*	*	*	*	*	*	*	*
Lactose	*	*	*	*	*	*	*	*	*	*
Raffinose	A	*	A	*	A	*	*	A	*	A
Sucrose	A	A	A	A	A	A	A	A	A	A
Arabinose	*	A	*	*	*	*	*	*	*	*
Maltose	A	A	A	A	A	A	A	A	A	A

Key: *No growth or activity.
**Expressed as per cent of the end product of glucose metabolism.
†Growth or activity.
A, Acid.

The cultural characteristics of 10 strains of the gram-positive, nonsporulating bacilli cultivated from 9 separate carious lesions are found in Table III. These organisms were nearly homogeneous in their reactions. On tomato juice agar they produced the smooth colonial form typical of oral lactobacilli. They were fairly small bacilli which did not become particularly pleomorphic in old cultures or under drastic growth conditions. They were obligatorily anaerobic when first isolated, but grew subsequently in the presence of atmospheric oxygen. They survived in Douglas' broth at pH 3.0 for 48 hours or more, and grew in trypticase soy broth or on tomato juice agar at pH 5.0. They were homofermentative, for lactic acid constituted at least 80 per cent of the end products

of their utilization of glucose. They did not liquefy gelatin. They did not produce acids from lactose. They acted on glucose, sucrose, and maltose in standard fermentation tubes to produce acids, and, after a long period of incubation, a very small amount of gas. Strains 1, 3, 5, 8, and 10 produced acids from raffinose, while the others were not able to do so. Strain 2 produced a small amount of acid from arabinose, but the other strains were not able to do so.

The acidogenic ability of this group of gram-positive, nonsporulating bacilli indicates that it belongs to the genus Lactobacillus. This conclusion is supported by the homofermentation of glucose to produce lactic acid. The failure of this group of bacteria to ferment lactose, however, confuses the species designation, since most of the common oral lactobacilli will ferment this sugar. The ability to ferment lactose is also characteristic of a group of lactobacilli that are not normal inhabitants of the mouth.[21]

TABLE IV

CHARACTERISTICS OF THE ANAEROBIC GRAM-POSITIVE FILAMENTOUS ORGANISMS FROM DENTINAL CARIES

	10 STRAINS
Terminal acidity in thioglycollate broth	5.0-5.2
Production of conidia	*
Growth on tomato juice agar at pH 6.0	†
Growth in Douglas' broth	*
Growth on blood agar	‡
Formation of aerial hyphae	*
Gelatin liquefaction	*
Coagulated serum slants {Growth	†
Coagulated serum slants {Liquefaction	*
Production of color changes to intact dentin	†
Acid-fast	*
Glucose	A**
Maltose	A
Sucrose	A
Mannitol	*
Lactose	*

Key: A Acid.
* Negative results.
** A small amount of gas was produced after 2 weeks or more of growth.
† Positive results.
‡ Questionable results.

The cultural characteristics of 10 strains of the filamentous microorganisms, cultivated from 9 carious lesions, are found in Table IV. The organisms grew with difficulty when initially isolated, but after subculturing in thioglycollate broth they grew well after 4 or 5 days incubation at 37.5°C. After subculturing for several months they grew regularly within 24 to 48 hours, producing a characteristic granular growth. After 48 hours incubation these granules became larger and more numerous. Prolonged cultivation produced a diffuse growth and loss of granularity.

The filamentous organisms were never grown successfully in Douglas' broth. Scant growth was obtained occasionally on rabbit blood agar. They were anaerobic and gram-positive. All attempts to induce the organism to form aerial hyphae or conidia were futile. They were not acid-fast. Terminal acidities between pH 5.0 and 5.2 were produced in thioglycollate broth containing

1 per cent glucose. Small amounts of gas were produced in this medium if the cultures were incubated for 14 days or more. A microscopic examination of this organism showed a filamentous form which branched regularly but not often (Fig. 1*A* and *B*). Its filaments were small in diameter.

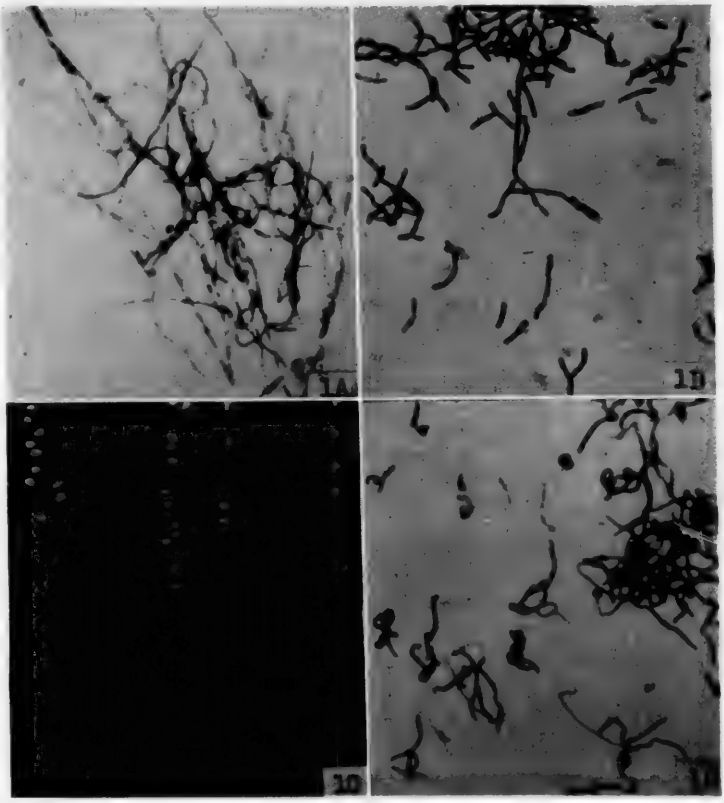


Fig. 1.—*A*, The cellular morphology of strain 4 of the actinomyces grown in thioglycollate broth for 48 hours. Gram stain, ×970. *B*, The cellular morphology of strain 4 of the actinomyces grown anaerobically on tomato juice agar (pH 6.0) for 48 hours, showing branching. Gram stain, ×1050. *C*, The colonial morphology of strain 9 of the actinomyces grown anaerobically on tomato juice agar (pH 6.0) for 72 hours, ×10. *D*, The cellular morphology of strain 9 of the actinomyces grown anaerobically on tomato juice agar (pH 6.0) for 96 hours, showing the pleomorphic and corkscrew forms. Gram stain, ×970.

After subculturing in thioglycollate broth for several months, these organisms acquired the capacity to form distinct colonies when cultured anaerobically upon tomato juice agar adjusted to pH 6.0. The colonies produced by various strains were small and granular and had an indefinite margin. They were heaped up to a moderate peak, papillate, rather off white in color, granular in consistency, not adherent to medium and could be emulsified but not easily. They had a fimbriate margin and were up to 2 mm. in diameter (Fig. 1*C*). When grown on solid media the mycelia were shorter and much more pleomorphic than when grown in broth (Fig. 1*D*).

They did not liquefy gelatin or coagulated serum. A distinguishing characteristic of the filamentous microorganisms was their ability to form a dark brown pigment on intact dentin exposed to them, but they were not able to do so upon other proteins, nor upon media not containing proteins. They produced acid from glucose, maltose and sucrose, but not from mannitol or lactose in thioglycollate broth (glucose-free).

The morphological and cultural characteristics of the gram-positive, anaerobic, filamentous organisms are uniform and correspond to those of the genus Actinomyces. However, a species designation presents certain difficulties. The colonial morphology of this group on solid media does not resemble the description of either *A. bovis* or *A. israeli*.[21] This conclusion has been substantiated by a direct comparison with five strains of actinomyces isolated from cases of human actinomycosis. On the other hand, in their failure to produce aerial hyphae, and in their fermentation reactions these organisms resemble *A. bovis* more closely than any other defined species.

DISCUSSION

In the many attempts to determine the etiological agent or agents of dental caries during the last 60 or 70 years, too little effort has been directed toward determining the nature of the bacterial flora in the deeper layers of dentinal caries. This area is particularly suitable for study since it is not easily contaminated adventitiously by bacteria from the oral cavity. The microorganisms present should function in the destructive process since, presumably, they must have gotten to their location by invading the dentin. In order to invade, it is presumed that they must change the character of normal intact dentin in some way, even if they invaded exclusively by the tubules. It is not known to what extent nutritional elements such as carbohydrates, etc., are able to diffuse into this area; hence, it may be postulated that these bacteria are able to gain at least part of their sustenance from the organic matrix of dentin during the process of invasion.

Those who have attempted to determine the bacterial flora of the deeper layers of the dentinal lesion have not always described clearly their methods for obtaining the samples. Neither is it usually possible to determine exactly from what level the samples were taken. The method of sampling and the level at which the samples were taken are especially important, for contamination of the advancing front from the outer periphery of the lesion while taking the sample would lead to erroneous conclusions. On the other hand, methods have been used which could kill the bacteria in the area from which the samples were taken. The utilization of mechanical cutting devices to remove the superficial layers of dentin adjacent to the area sampled might produce sufficient heat to kill the bacteria present. This would be especially true if the advancing carious front were approached from the pulpal side. Furthermore, when utilizing this method, difficulty is experienced in determining the exact location of the advancing front. If the advancing carious front in dentin were approached from the pulpal side it seems quite likely that the samples might be taken from an

area which no microorganisms had invaded. Such methods could easily lead to the conclusion that the advancing front was sterile. On the basis of the sampling methods, therefore, it is difficult to compare the results obtained by the various investigators.

In addition to the variety and limitations of the sampling methods used, the cultural methods applied have not always been conducive to the growth of such bacteria as might be expected in the advancing carious lesion of dentin. Some workers have utilized methods limited to certain bacterial species. Others have used only aerobic methods. Very few, if any, have attempted to determine specifically whether or not proteolytic bacteria are present.

The method used in this study was designed to give a balance between the possibility of affecting the bacterial flora at the time the sample was taken and the likelihood of contaminating the area from which it was taken. Furthermore, the cultural procedure used was designed to recover the maximum numbers of bacteria present while differentiating between the aciduric and proteolytic types. In this way, only three characteristic groups of microorganisms were obtained, with the isolation of only an occasional bacterial form that could be considered as a contaminant. It does not seem likely that only these three groups of bacteria would have been obtained regularly if the method of obtaining the sample had been contaminating the area from which it was taken. The bacterial flora which could be cultured was quite distinct, quantitatively and qualitatively, from that found in the area most likely to have caused contamination, namely, the superficial levels where there was the heaviest concentration of bacteria.[16]

Some difficulty was experienced in placing these three groups of microorganisms in any defined species, but there can be little doubt that they belonged to the genera in which they were placed. Definitive classification was made more difficult by changes in their growth pattern after isolation from the carious lesion. The colonial morphology and growth pattern of the filamentous microorganisms changed drastically when they were isolated in pure culture. The lactobacilli exhibited the same behavior by acquiring the ability to grow aerobically. The classification of the cocci was confused by several factors, the most important of which were their differences in catalase production and aciduric qualities.

However, it is felt that the definition of the role of these three groups of microorganisms in dentinal caries did not necessarily depend upon their classification, but rather upon the reactions which they were able to effect. It must also be pointed out that the reactions of these organisms which we have described are those of organisms growing in pure culture, and away from the environment of carious dentin and from the influences of others of the group. While our studies contribute certain points to our knowledge of the flora of the dentinal lesion and to the reactions occurring there, they do not, by any means, give us the complete picture of what is occurring in such a complex situation. Two groups, the lactobacilli and actinomyces, were able to produce sufficient acids in vitro to decalcify enamel or dentin. Under similar conditions, the aciduric

cocci did not produce as much acid as the other groups. In addition, there remains a large group of cocci isolated from the advancing front which were neither aciduric, nor proteolytic against the more resistant proteins such as coagulated egg or decalcified dentin. Upon the basis of their reactions one might be led to conclude that they do not function in effecting the carious process, if rapid acidogenesis or proteolysis are the necessary prerequisites. Very little is known specifically about the rate of the reactions occurring in dentinal caries. It seems logical, however, to conclude that some of the reactions which produce changes in the organic matrix of dentin during caries are rather slow. Certainly these reactions cannot be considered in terms of 48 or 72 hours duration. In view of this assumption, the cocci should be investigated further in an attempt to determine the rate and type of their reactions before concluding that they can exist in close proximity to the advancing bacterial front without taking part in the carious process.

CONCLUSIONS

By the method of sampling and culturing employed, only three groups of microorganisms were isolated regularly from the advancing carious lesion in dentin, namely, cocci, lactobacilli, and actinomyces. Over 90 per cent of the count was made up of gram-positive cocci. Under anaerobic conditions, half of these were caseinolytic but none were active against more resistant proteins such as coagulated egg or decalcified dentin. Three per cent of the cocci were aciduric. Of eight strains studied in detail, three were micrococci and five were enterococci. Lactobacilli could be isolated only by anaerobic cultivation, under which condition they comprised, on the average, 5 per cent of the cultivable organisms. Ten strains, studied in detail, gave nearly homogeneous reactions, which were, however, not identical with those of any described species.

Anaerobic filamentous microorganisms were obtained regularly but their relative abundance could not be determined because they did not form discrete colonies in primary cultures. The characteristics of ten strains of these organisms were homogeneous and typical of the genus Actinomyces, although they did not agree exactly with the description of any one of the defined species. These actinomyces acted upon decalcified dentin to produce a dark-brown pigment resembling that often seen in natural caries. No pigment was produced from other proteins.

REFERENCES

1. Miller, W. D.: The Microorganisms of the Human Mouth, Philadelphia, 1890, S. S. White Dental Mfg. Co.
2. Goadby, K. W.: The Mycology of the Mouth, London, 1903, Longmans, Green and Co.
3. Gies, W. J.: Chemical Studies of the Relationship of Oral Microorganisms to Dental Caries, *J. Allied Den. Soc.* **10**: 137, 1915.
4. Gies, W. J.: Retrospect and Prospect, *J. Allied Den. Soc.* **10**: 464, 1915.
5. Gies, W. J., Hull, L. M., and Mullikin, J. C.: Tests of Some Biochemical Activity of Oral Bacteria, *J. Allied Den. Soc.* **10**: 459, 1915.
6. Gies, W. J., and Kligler, I. J.: I. A Biochemical Study and Differentiation of the Oral Bacteria, With Special Reference to Dental Caries, *J. Allied Den. Soc.* **10**: 141, 1915.
7. Gies, W. J., and Kligler, I. J.: II. A Biochemical Study and Differentiation of the Oral Bacteria, With Special Reference to Dental Caries, *J. Allied Den. Soc.* **10**: 282, 1915.

8. Gies, W. J., and Kligler, I. J.: III. A Biochemical Study and Differentiation of the Oral Bacteria, With Special Reference to Dental Caries, *J. Allied Den. Soc.* **10**: 445, 1915.
9. Clarke, J. K.: On the Bacterial Factor in the Aetiology of Dental Caries, *Brit. J. Exper. Path.* **5**: 141, 1924.
10. Bunting, R. W., and Palmerlee, F.: The Role of Bacillus acidophilus in Dental Caries, *J. A. D. A.* **12**: 381, 1925.
11. Bunting, R. W., Nickerson, G., and Hard, D. G.: Further Studies of the Relation of Bacillus acidophilus to Dental Caries, *D. Cosmos* **68**: 931, 1926.
12. MacLean, I. H.: The Bacteriology of Dental Caries, *Proc. Royal Soc. Med. Odont. Soc.* **20**: 873, 1927.
13. Canby, C. P., and Bernier, J. L.: Bacteriologic Studies of Carious Dentin, *J. A. D. A.* **23**: 2083, 1936.
14. Sognnaes, R. F., and Wislocki, G. B.: Enamel and Dentine Undergoing Carious Destruction, *Oral Surg., Oral Med., & Oral Path.* **3**: 1283, 1950.
15. Thoma, K. H.: Oral Pathology, St. Louis, 1941, The C. V. Mosby Company.
16. Burnett, G. W., and Scherp, H. W.: The Distribution of Proteolytic and Aciduric Bacteria in Saliva and in the Carious Lesion, *Oral Surg., Oral Med., & Oral Path.* **4**: 469, 1951.
17. Somogyi, M.: A New Reagent for the Determination of Sugars, *J. Biol. Chem.* **160**: 61, 1945.
18. Schales, O., and Schales, S. S.: A Simple Method for the Determination of Glucose in Blood, *Arch. Biochem.* **8**: 285, 1945.
19. Barker, S. B., and Summerson, W. H.: The Colorimetric Determination of Lactic Acid in Biological Material, *J. Biol. Chem.* **135**: 535, 1941.
20. Isaacs, A., and Scouller, J. M.: Catalase Production Among the Coccaceae, *J. Path. and Bact.* **60**: 135, 1948.
21. Breed, R. S., Murray, E. G. D., and Hitchens, A. P.: Bergey's Manual of Determinative Bacteriology, ed. 6, Baltimore, 1948, Williams and Wilkins Company.

HAMSTER CARIES

The Effects of Three Different Dietary Sugar Levels and an Evaluation of Scoring Procedures

DAVID F. MITCHELL, D. S. CHERNAUSEK, AND EDITH ZAK HELMAN

University of Minnesota, School of Dentistry, Minneapolis 14, Minn.

THIS IS an effort to establish a caries-producing diet for the hamster that will yield reproducible caries scores in a short period of time. Shafer[1] showed that sucrose, a simple carbohydrate, has a greater caries-producing potential than starch, a carbohydrate of higher molecular weight. It was proposed to evaluate the effects of different percentages of sucrose in the caries-producing diet. Certain data obtained were treated by various scoring procedures.

METHOD

The animals used were from an inbred colony. After weaning they were maintained on a diet of laboratory chow supplemented with fresh carrots and hamburger until they were approximately 30 days old. They were placed then on the experimental diets* for 104 days. Only littermate animals were used. Three animals of each sex in a litter were selected by lot and distributed among the experimental groups. Each experimental group consisted of fifty-six animals (28 ♀, 28 ♂). Only four animals failed to survive the experimental procedures. After the animals were sacrificed, the carious lésions were outlined on the score sheets of Keyes.[2] Certain statistics derived from these scores will be considered subsequently.

RESULTS

Sugar Level Study.—The mean weights of the female and male animals of the experimental groups were calculated weekly. Typical weight curves for animals of this age were produced. The mean terminal weights of the Group III animals were lower than those of Groups I and II. Mean terminal weights: Group I, ♀ 123.6, ♂ 122.2, Group II, ♀ 118.5, ♂ 121.3, and Group III, ♀ 107.0, ♂ 110.1.

The caries scores are tabulated in Table I, on the basis of occlusal scores (occlusal area times depth factor; possible total of 132). The reasons for using these statistics will be developed subsequently.

The question of the existence of a sex factor in hamster caries has arisen repeatedly.[3, 4] The scores of the male and female animals of each of the experimental groups were tested statistically by the difference between mean

This work was supported in part by a grant from the Graduate School, University of Minnesota.

Received for publication June 14, 1951.

*Group I whole wheat flour 30 per cent, whole powdered milk 30 per cent, corn starch 20 per cent, confectionary sugar 15 per cent, alfalfa meal 4 per cent, and NaCl 1 per cent
Percentages of above items respectively:
Group II 25, 25, 15, 30, 4, 1.
Group III 20, 20, 10, 45, 4, 1.

TABLE I

OCCLUSAL CARIES SCORES

GROUP	NO. OF ANIMALS	MEAN SCORE	STANDARD DEVIATION
I ♀	28	29.79	7.08
♂	27	46.85	13.27
♀ + ♂	55	38.16	13.60
II ♀	26	29.94	13.21
♂	28	49.08	15.68
♀ + ♂	54	39.85	17.44
III ♀	26	38.31	13.23
♂	27	51.63	15.75
♀ + ♂	53	45.09	16.03

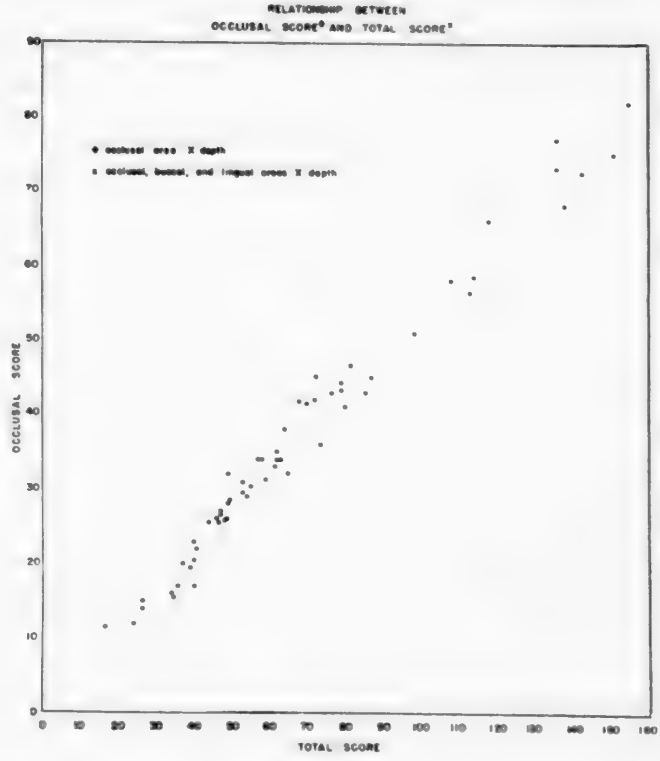


Fig. 1.

scores. The "t" values and their "probabilities" are: Group I, 5.86 (.001), Group II, 4.74 (.001), and Group III, 3.26 (.003). The males experienced a significantly greater degree of caries.

Conceivably, each experimental group can be considered as three distinct subgroups (female, male, and combined). The "t" values and their "probabilities" resulting from the statistical tests are listed in Table II. No significant differences at or below the .01 level were found except when Group I females were compared with Group III females.

TABLE II

CARIES SCORES—"t" VALUES AND "PROBABILITIES"

GROUPS COMPARED	FEMALES	MALES	COMBINED
I vs II	.05 (>.656)	.56 (.553)	.54 (>.656)
I vs III	2.92 (.007)	1.18 (.240)	2.40 (.023)
II vs III	2.24 (.036)	.59 (.553)	1.6$ (.120)

Caries Scoring Evaluation.—It seemed desirable to reduce, if possible, the effort required for the scoring method originally advocated by Keyes.[2] Two factors, other than time involved, make a revision desirable: (a) when a "cavity" occurs on more than one surface, two "volumes" are summated for that particular cavity; and (b) in our experience, buccal and lingual cavities rarely occur without coalescing with the occlusal caries (if sufficient caries for experimental purposes is present). Using approximately one-third of the animals,

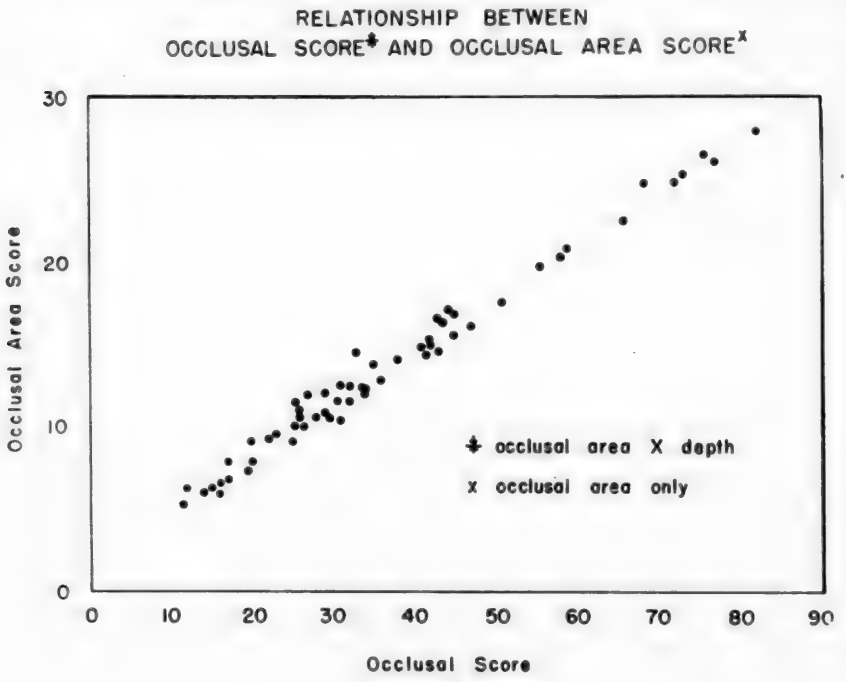


Fig. 2.

a scatter diagram (Fig. 1) of the relationship between total score (possible, 282) and occlusal score (possible, 132) is plotted. It is apparent that a positive relationship exists. Using the same animals, the relationship between occlusal score (area × depth factor) and occlusal area alone (possible, 44) is shown in Fig. 2. A very close relationship is apparent. A perfect relationship would be shown by a straight line.

The value of littermate distribution of animals in experimental groups is generally ignored by most investigators. Fig. 3 is a scatter diagram of littermates of Groups I and II. Although a clean cut relationship is not present, the dots do seem to follow a linear pattern, indicating a degree of similarity of caries experience of the littermates of the two groups. The correlation coefficient for these data is 0.58.

DISCUSSION

A definite sex difference in regard to caries has been shown to be present in these experimental animals. This observation holds only for animals that underwent the experimental procedures during a specific period of their lives (30-130± days of age). It seems ill advised to ignore or neglect the sex factor. The grouping of the two sexes to make a larger number for mathematical purposes should not be encouraged.

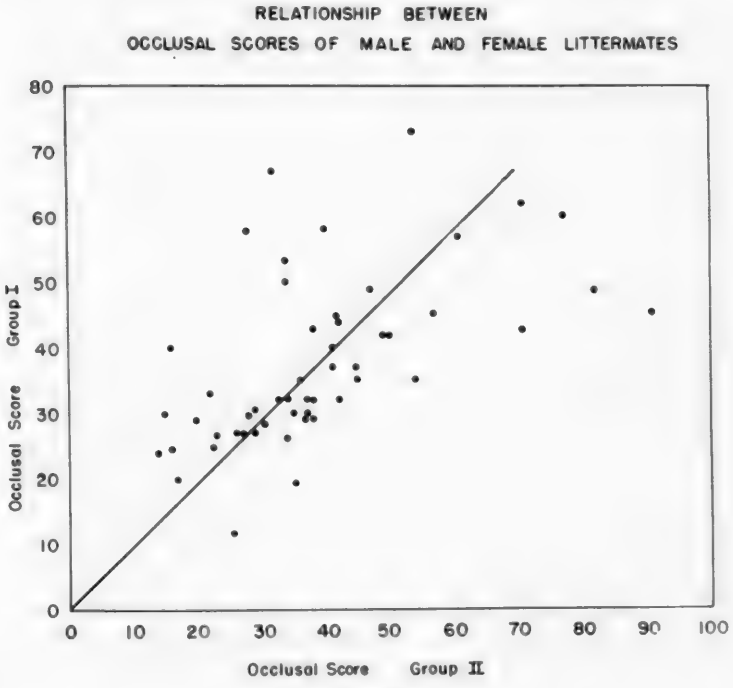


Fig. 3.

Table II illustrates a significant difference at the .01 level between the females of Groups I and III, but not in the male grouping or in the combined grouping. Although the 45 per cent sugar diet (Group III) was associated with higher caries scores than were the lower sugar level diets used in this study, the differences between Groups I, II and III were not graded according to sugar level, nor were they statistically significant when male, or combined

male and female scores were compared. There does not appear to be a great advantage in using 45 per cent sugar rather than 30 per cent or 15 per cent sugar in a caries-producing diet.

When sufficient caries is present, the occlusal score is as good a statistic as total score. Likewise, the depth factor is of small consequence. It is of paramount importance to have a sufficiently large number of animals in the experimental groups as the variation among individual animals is considerable.

It seems that the use of littermate groupings is desirable. Fig. 3 arises from an unusual situation—since Groups I and II produced similar caries scores (Table I), an unusual opportunity to evaluate this factor was present as each point was determined by a pair of animals from the same litter, one from Group I and one from Group II.

CONCLUSIONS

1. The 45 per cent dietary sugar level was associated with slightly more caries and lower terminal weights in the animals than were the other two diet levels of sugar used, 30 and 15 per cent.

2. More caries was experienced by the male than the female animals. Sex groups of animals should be considered separately.

3. The use of occlusal area only, gave a simple method and reliable scores for evaluating caries experience.

4. Evidence is offered to show that the use of littermate grouping of experimental animals has value in this type of study.

REFERENCES

1. Shafer, W. G.: The Caries-Producing Capacity of Starch, Glucose, and Sucrose Diets in the Syrian Hamster, *Science* **110**: 143, 1949.
2. Keyes, P. H.: A Method of Recording and Scoring Gross Carious Lesions in the Syrian Hamster, *J. D. Res.* **23**: 427, 1944.
3. Ibid: Dental Caries in the Syrian Hamster. IV. The Effect of Gonadectomy on Caries Activity and Body Growth, *New York D. J.* **18**: 172, 1948.
4. Granados, H., Glavind, J., and Dam, H.: Observations on Experimental Dental Caries. X. Difference of Sex in Caries Susceptibility? *J. D. Res.* **29**: 194, 1950.

SYMPATHICOTONY OF THE SALIVARY GLANDS AS A CAUSE OF DENTAL CARIES

WERNER RATHJE

Kaiser Wilhelm Institut für Biochemie, Tübingen, Germany

THE MODEL experiments herein described were undertaken to investigate the problem of why decomposition of enamel occurs in mouths of susceptible individuals and not in those of resistant individuals.

Lumps of white bread inoculated with acidogenic bacteria (from an incubated dilution of bread chewed by an individual with dental caries) were placed in Petri dishes containing agar solutions of different concentrations and viscosity, enough sodium acetate to maintain the average pH (6.5) and buffer capacity (n/500 acetic acid) of saliva, and methyl red as an indicator. The viscosity of the agar solution was measured in a 1 c.c. capacity viscometer in which water had a percolating time of 3.2 seconds.

After incubation of 16 hours to 4 days:

1. All bread lumps in the gelatiniform agar solutions gave acid reactions.
2. All bread lumps in agar solution of low viscosity (percolating time at 37° C. 10 seconds) remained neutral in reaction.
3. Bread lumps in agar solution of low viscosity (percolating time at 37° C. 10 seconds) but which had been enclosed within glass rings gave acid reactions.
4. Larger bread lumps (1/5 inch) gave acid reactions sooner than small bread lumps (1/25 inch) in medium viscosity agar solutions (percolating time, at 37° C. for 25 seconds).
5. Bread lumps to which 2 per cent cane sugar had been added produced so much acid in the media of high viscosity that large areas of the agar surrounding the lumps turned red.

These findings demonstrate that acids originating in the bread, particularly in the presence of soluble sugar, are neutralized and eliminated more completely in more liquid (less viscous) agar solutions, with smaller bread lumps and if the convection and diffusion of the agar solution is not impeded.

The presumption may be made that when carbohydrate-containing food remains adherent to the teeth the acids which are formed are neutralized and eliminated by saliva. In individuals with high resistance to caries this eliminating mechanism may be so complete that the enamel is not dissolved, while in the highly caries susceptible this salivary activity may be negligible. Among the generally accepted observations justifying this presumption are: (1) When caries occurs on both approximal surfaces between two teeth in which food particles are retained it is arrested on one surface if the other tooth is extracted, (2) undermining caries is arrested when the surrounding enamel impeding neutralization and elimination of acids is removed, (3) Caries appears at the areas where food is retained, i.e., interstices and fissures, (4) The lower incisors

Received for publication, February 27, 1951; revised by author, August 21, 1951.

are least commonly attacked by caries and they are bathed by saliva capable of neutralizing and eliminating acids, (5) In cases of reduced salivary production caries is greater, (6) Eating soluble and quickly fermentative oligosaccharides (glucose and sucrose) results in greater caries susceptibility.

RELATION BETWEEN CARIES SUSCEPTIBILITY, VISCOSITY, AND SPEED OF SALIVARY SECRETION

To further evaluate this theory of the relationship between caries activity and saliva viscosity and flow, the viscosity of saliva and its speed of secretion were measured in 101 individuals with as little external stimulation as possible.

TABLE I

VISCOSITY* AND SPEED OF SALIVA SECRETION AT VARIOUS DEGREES OF CARIES SUSCEPTIBILITY

PATIENT	PERCOLATING TIME IN VISCOMETER (SECONDS)	SPEED OF SECRETION (C.C./10 MINUTES)
Very strong caries susceptibility (nearly all teeth destroyed lately)		
L. S.	**	**
M. N.	**	**
M. S.	**	**
S. B.	**	**
W. L.	**	**
V. R.	**	**
L. K.	400	0.2
W. H.	240	1.4
M. S.	190	1.0
M. W.	180	0.4
Mean	250	0.8
P. E.$_m$	±51	±0.3
Strong caries susceptibility (3 to 8 teeth filled or extracted, more than 3 new caries lesions)		
P. K.	360	0.4
Dr. G.	290	1.8
K. H.	276	0.8
L. S.	240	0.4
A. H.	220	0.6
H. K.	215	0.7
W. H.	207	0.9
E. D.	183	1.2
F. G.	170	0.9
E. B.	150	1.5
L. L.	130	1.1
H. Z.	120	0.9
Dr. R.	120	0.9
H. O.	105	2.0
L. M.	104	0.4
P.	97	2.2
H. Z.	95	0.9
Dr. S.	87	1.5
L. V.	80	5.5
A. F.	80	1.0
S. M.	63	3.0
H. E.	60	0.4
G. Z.	60	4.0
J. W.	53	2.5
T. H.	48	1.2
H. P.	46	3.0
B. H.	18	4.5
Mean	136	1.6
P. E.$_m$	±17	±0.3

TABLE I—CONT'D

PATIENT	PERCOLATING TIME IN VISCOMETER (SECONDS)	SECRETION SPEED OF (C.C./10 MINUTES)
Medium caries susceptibility (3 to 15 teeth filled or extracted, 1 to 3 new caries lesions) 42 patients		
Extremes	11 & 90	0.4 & 4.1
Mean	40	2.9
P. E.$_m$	10	0.5
Caries resistance (2 teeth, at the most, filled or extracted, no new caries lesion)		
K. S.	45	2.0
H. B.	42	1.2
H. K.	35	2.3
F. B.	30	2.7
S. B.	29	1.7
W. W.	28	3.3
H. M.	25	4.1
H. K.	22	3.6
A. S.	20	7.0
H. C.	20	4.0
P. T.	19	2.1
R. B.	17	3.8
G. B.	15	3.4
W. S.	14	2.7
R. H.	11	4.8
S.	11	4.5
B. F.	9	3.2
W. R.	9	3.2
E. T.	7	5.3
W. E.	5	10.3
Dr. S.	5	4.0
M. H.	4	7.2
Mean	24	3.9
P. E.$_m$	±6	±0.4

*The viscosity of the saliva decreases, as is known, when it is stirred, and therefore also in viscosity determination, so that lower viscosities will be found by repeated measurings. These results were obtained from the first viscosity measurings.

**No saliva available because of a too slow secretion, even in a long period (15 minutes).

The results (Table I) indicate that on the average the viscosity of the saliva and its speed of flow are considerably lower in caries-resistant than in caries-susceptible individuals.

The mechanism of dental caries may be explained on the basis of acids being formed from carbohydrates by acidogenic bacteria; in resistant mouths the acids being formed only to a limited extent, or neutralized by sufficient and thin saliva, while in susceptible mouths there is insufficient salivary neutralization and elimination of acids which are formed to a greater extent by the prevailing acidogenic bacteria.* In cases of caries susceptibility the acid concentration in carbohydrate-containing food retained between the teeth may reach a pH as low as 3.0 and the enamel may be dissolved rapidly.

In order to obtain the results of Table I, it was of great importance to get the saliva with as little stimulation as possible (draining from the slightly opened mouth while the person was mentally preoccupied), since the imagination of eat-

*This large augmentation of the acidogenic bacteria in the food remnants containing carbohydrates which were not sufficiently neutralized by too little and too viscous saliva, seems to explain the results of the salivary lactobacillus test in which a parallelism is assumed to exist between caries susceptibility and number of acidogenic bacteria in saliva which was obtained during the chewing of paraffin.

ing food, the mental concentration upon saliva production with all possible speed, and, in particular, chewing motions and rinsing with acid solutions, effected a decrease of viscosity, and an increase of the speed of secretion. These psychic and physical influences on the quantity and quality of the saliva may be the reason why the simple connection between caries susceptibility, viscosity, and speed of saliva secretion was not recognized hitherto, and that in the literature of this field a clash of opinions is found.

EFFECTS OF IONIZING RADIATION OF ORAL STRUCTURES

I. Pilot Studies on Dental Caries in the White Rat

C. A. SCHLACK, AND F. ELLINGER
WITH THE TECHNICAL ASSISTANCE OF C. E. FRANER, V. G. BERZINSKAS, W. D. CLARK, AND D. E. TETER

From the Naval Medical Research Institute, Bethesda, Md.

AS EARLY as 1905 Tribondeau and Recamier[1] mentioned radiation-induced changes of teeth. In subsequent years, effects of ionizing radiation on teeth and supporting structures did not attract much interest until the report of Blum[2] in 1924 on "osteomyelitis of the mandible and maxilla" reactivated interest in the subject. Following Blum's report we find clinical[3] as well as experimental[4] reports indicating the sensitivity of the growing tooth to ionizing radiation. But ionizing radiation affects not only the growing tooth and the tooth-anlage, but also the adult tooth as shown by Gotthard,[5] Lüdin and Muller,[6] and especially by del Regato.[7] Superficial caries of the neck of the tooth, discoloration and wearing away of the incisal edges or occlusal surfaces of teeth have been observed, leading to dental pulp and periapical infectious involvements and subsequently to the dreaded jaw necrosis.[2, 8] Radio-osteonecrosis of the jaws has been recognized as one of the major complications of the successful radiological treatment of malignant tumors of the oral cavity. Experience has established the fact that this process is favored by the presence of diseased teeth in the field of irradiation. But it also appears that teeth which on clinical or radiological examination appear healthy, may become foci of infection and the starting point of jaw necrosis. Opinions about the etiologic mechanism of this pathologic process vary widely: direct effect on osteoblasts and cementoblasts of the dental periosteum and osteoblasts of the alveolar process, interference with normal circulation, especially in aged individuals exhibiting osteoporotic bone changes, and signs of maxillary arteriosclerosis have been taken into consideration as well as changes with quantity and quality of saliva induced during radiation therapy.[9*] In view of the afore-mentioned facts and opinions it is considered necessary "Whenever deep X-ray therapy is to be directed to lesions of the mouth that any teeth through which the beam will pass should be removed before treatment is started"[10] and that extraction of teeth in an irradiated area should possibly be omitted entirely, or, if unavoidable, should be done with the utmost care.[10, 11]

The effect of ionizing radiation on teeth and their supporting structures is, however, not only extremely important in radiation therapy, but has acquired also considerable interest from the point of view of military medicine and civilian defense in view of the severe cases of jaw necrosis observed in survivors of atomic bomb explosions in Japan.[12]

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Navy or the naval service at large.

Received for publication, April 26, 1951.

*Finally the possibility of induction of chemical mutations in the bacteria composing the oral flora deserves consideration. These mutans may release metabolic products harmful to teeth.

While there exists a satisfactory experimental background for the understanding of the growth inhibiting effect of ionizing radiation on teeth, no such material has been available so far with regard to the cariogenic action of ionizing radiation. The present study represents a first step in this direction.

METHOD OF PROCEDURE

One hundred seventy-two white rats, representing 43 litters, from the Naval Medical Research Institute's dental caries susceptible strain, form the biologic material of this study. At the age of three weeks these were divided into two littermate groups, with equal numbers of males and females within each litter of each group and no significant difference between the mean weight of rodents in each subgroup within each group. Under temperature and humidity control and vermin-free conditions, the treatment of these groups was as follows:

Group I (Composed of 24 litters).—

Subgroup *A* received a coarse corn cariogenic diet* and distilled water taken ad libitum[13] (control).

Subgroup *B* received the same dietary regimen as *A* but in addition the rodents were irradiated with 200 r in one total body dose.

Group II (composed of 19 litters).—

Subgroup *A* received Purina Lab Chow (stock diet) and distilled water taken ad libitum (control).

Subgroup *B* received the same dietary regimen as *A* but the animals were irradiated with 200 r in one total body dose.

Radiation factors were: 200 K.V., 25 ma, 50 cm. distance. No added filtration. HVL = 0.75 mm. Cu. Intensity 4.25 r per minute. The x-ray dose represents about one-third of the LD_{50} per thirty days for adult rats. This dose was selected as an effective† but nonlethal dose for adult specimens of this series.

After 100 days on the dietary regimen described, the rodents were killed and their teeth examined for dental caries incidence and extent in accordance with a standard procedure previously reported.[14] Weight gains were also observed.

In order to reduce the introduction of variables to a minimum, and to emphasize the effect of irradiation on dental caries in these animals, analyses of results were made only on those litters in which none of the rodents died as a result of irradiation. Thus 18 litters of 72 rodents on the dental cariogenic dietary regimen and 17 litters of 68 rodents on the Purina Lab Chow regimen were used in the analyses.

*Quaker Oats coarse hominy grits have been used instead of coarse corn throughout all of the rodent dental caries studies. Otherwise the dietary is the same as that used by Hoppert.

†Effective in the sense that this dose affects the blood-forming tissues and the body weight of the animals.

The methods of statistical analyses of results for Group I were those employed in other studies[13, 15] as well as the Chi Square Tests for extent of caries. For Group II on caries incidence and extent, however, "t" tests for significance were used because the sensitivity of the analyses of variance could not be applied due to the number of rodents with no dental caries, as is expected with this dietary regimen.

TABLE I

WHITE RATS ON A DENTAL CARIOGENIC AND PURINA LABORATORY CHOW DIETARY REGIMEN WITH DISTILLED DRINKING WATER, AND IRRADIATED 200 r IN ONE TOTAL BODY DOSE

	NUMBER	LITTER NUMBER	SEX	INITIAL WEIGHT	FINAL WEIGHT	WEIGHT GAIN	CARIES INCIDENCE	CARIES EXTENT SCORE 1	SCORE 2	SCORE 3
CARIOGENIC DIET (CONTROL)										
Total	36	18	18M 18F	1080	7466	6386	543	70	28	445
Mean				30.00	207.39	177.39	15.08	1.94	.78	12.36
St. Dev.				6.84	41.26	38.69	5.25	2.29	1.17	5.12
PURINA LAB CHOW (CONTROL)										
Total	34	17	17M 17F	881	10022	9141	66	61	3	2
Mean				25.91	294.76	268.85	1.94	1.79	.09	.06
St. Dev.				7.26	81.96	74.67	1.91	1.82	.37	.33
CARIOGENIC DIET + 200 r IN A SINGLE TOTAL BODY DOSE										
Total	36	18	18M 18F	1073	6456	5383	644	63	34	547
Mean				29.81	179.33	149.53	17.89	1.75	.94	15.19
St. Dev.				5.99	29.55	26.98	5.50	1.53	1.32	5.19
PURINA LAB CHOW + 200 r IN ONE SINGLE BODY DOSE										
Total	34	17	17M 17F	837	8504	7667	111	76	6	29
Mean				24.62	250.12	225.50	3.27	2.24	.18	.85
St. Dev.				6.51	64.56	73.41	5.39	2.32	.62	3.86

Age, three weeks.
Duration of experiment, 100 days.

RESULTS

Influence of Diet and X-ray on Mortality.—It was noticed that 8 out of the 172 of the irradiated animals (5 per cent) died, while no fatalities occurred among the unirradiated rats; 6 of these fatalities occurred in the cariogenic and 2 in Purina Lab Chow dietary subgroups. The respective deaths occurred on the following days of the experiment: 3, 12, 15, 17, 19, 37, 46, and 71. Of the dead animals, 6 were males and 2 females, and although this difference is not significant, it is in conformity with data in which a greater sensitivity to radiation was demonstrated in the male.[16]

Influence of Diet and X-ray on Weight.—Inspection of Table I reveals that irradiated rodents gained less weight on the average than those which were

not irradiated irrespective of the dietary regimen, at the 1 per cent level of probability by analysis of variance test. Furthermore the unirradiated animals on the dental cariogenic dietary also showed a less mean weight gain over the 100-day experimental period than did those on the Purina Lab Chow (1 per cent level of probability by "t" test).

Dental Caries Incidence and Extent.—In the survival litters of rodents receiving the cariogenic dietary regimen and 200 r (Group I, subgroup *B*) Table I indicates that the incidence of dental caries (mean of 17.89) was significantly higher than in the nonirradiated rodents (mean of 15.08) (Group I, subgroup *A*) at a 5 per cent level of probability by analysis of variance test. Females showed dental caries incidence in the irradiated (mean of 16.22) and the nonirradiated (mean of 13.67) not significantly different from that in the males (mean of 19.56 and 16.50 respectively). Examination of the extent scores reveals no significant difference between irradiated and unirradiated animals other than what may be attributed to an increase in incidence.

In the surviving litters of those rodents receiving the Purina Lab Chow regimen and 200 r (Group II, subgroup *B*) Table I indicates that there was no significant difference in the dental caries incidence, between these animals (mean of 3.27) and the nonirradiated animals (mean of 1.94) (Group II, subgroup *A*).

DISCUSSION

The foregoing data clearly indicate that combined influence of cariogenic diet and radiation produce an increasing incidence of caries. This observation represents to our knowledge the first experimental evidence of a caries facilitating influence of the x-ray which so far only has been inferred from the clinical observations mentioned in the introduction. At this point it appears unwise to enter into discussion of the possible mechanism involved in this process. It is hoped that future studies using larger doses of x-ray, locally applied over the head, will furnish the desired evidence on which the problem of the cariogenic action of ionizing radiation can be clarified.

SUMMARY

1. A pilot study involving carious susceptible rats of the Naval Medical Research Institute strain kept on a cariogenic and Purina Laboratory Chow dietary regimen and exposed at the age of three weeks to 200 r of x-ray (HVL = .75 mm. Cu) given as total body radiation in a single exposure is reported.

2. The following results were obtained: (a) In the group of irradiated animals kept on the cariogenic dietary regimen, a statistically significant increase of dental caries incidence was found, as compared to the unirradiated controls; (b) in the group of irradiated animals kept on Purina Laboratory Chow, no such results were obtained; (c) the analysis of the weight curves indicates a lesser gain of body weight of the irradiated animals irrespective of the dietary regimen.

The authors are indebted to Dr. John Flynn for his assistance in the statistical methods applied.

REFERENCES

1. Tribondeau, L., and Recaminer, A. C.: *Reunion Biologique de Bordeaux* **58**: 1031, 1905.
2. Blum, T.: *J. A. D. A.* **11**: 802, 1924.
3. Herbst, E.: *Fortschr. Zahnheilk.* **3**: 855, 867, 1927.
   Lacronique, G., Beal, G., and Goudeart, M.: *Rev. de stomatol.* **48**: 541, 1947.
4. Burstone, M. S.; *J. D. Res.* **29**: 230, 1950.
   Dale, P. P.: *J. D. Res.* **27**: 730, 1948.
   Dale, P. P.: *J. D. Res.* **29**: 678, 1950.
   Herold, K.: *Deutsche Monatschr. f. Zahnh.* **49**: 97, 1931.
   Leist, M.: *Ztschr. f. Stomatol.* **24**: 448, 452, 1924.
   Leist, M.: *Strahlentherapie* **24**: 268, 1926.
   Rosenthal, M.: *Am. J. M. Sc.* **193**: 495, 1937.
   Smith, H. A.: *J. A. D. A.* **18**: 111, 1931.
   Weinreb, M., Schour, I., Mendak, H., and Klauber, W. A., Jr.: *J. D. Res.* **28**: 633, 1949.
5. Gotthard, P. P.: *Fortschr. a. d. Geb. de Roentgenstrahlen* **30**: 139, 1923.
6. Lüdin, M., and Muller, O.: *Strahlentherapie* **56**: 644, 1936.
7. del Regato, J. A.: *Am. J. Roentgenol.* **42**: 404, 1939.
8. Immenkamp, A.: *Deutsche Zahnartztl. Wchnschr.* **38**: 549, 1935.
   Kanthak, F. F.: *J. A. D. A.* **28**: 1925, 1941.
   La Dow, C. S.: *Oral Surg., Oral Med., & Oral Path.* **3**: 582, 1950.
   Rahm, H.: *Strahlentherapie* **25**: 338, 1927.
9. Ochsen, H.: *Ztschr. f. Stomatol.* **32**: 145, 1934.
10. Daland, H.: *Radiology* **52**: 205, 1949.
11. Ryffel, W.: *Schweiz. Med. Wchschr.* **63**: 351, 1933.
    Zuppinger, A.: *Strahlentherapie* **72**: 562, 1943.
12. U. S. Strategic Bombing Survey: The Effects of Atomic Bombs on Health and Medical Services in Hiroshima and Nagasaki, Medical Division, U. S. Govt. Printing Office, Washington, D. C., 1947.
13. Schlack, C. A., Taylor, B. L., Gerende, L. J., Berzinskas, V. J., and Mullins, C. E.: *Oral Surg., Oral Med., & Oral Path.* **2**: 1208, 1949.
14. Schlack, C. A., Howell, S. R., and Taylor, B. L.: Project NM 008002, Report No. 5, Naval Medical Res. Inst., 17, July, 1947.
15. Schlack, C. A., Howell, S. R., Taylor, B. L., Berzinskas, V. J., and Aborn, M. H.: *J. D. Res.* **26**: 383, 1947.
16. Ellinger, F.: *Strahlentherapie* **44**: 1, 1932.

A HISTOCHEMICAL EVALUATION OF NORMAL AND INFLAMED HUMAN GINGIVAE

SAMUEL TURESKY, IRVING GLICKMAN, AND TAMARA LITWIN

Department of Oral Pathology and Periodontology Tufts College Dental School Boston, Mass.

PRESENT day knowledge regarding the histopathology of gingival disease has, for the most part, been derived from studies using staining techniques which reveal only morphological differentiation. Valuable as these have been, it has become increasingly apparent that such techniques are limited indices of the physicochemical nature of pathological tissue alterations involved in gingival disease. More recently histochemical staining techniques[1, 2] have been described which offer an approach to the study of gingival disease which is more comprehensive than that encompassed by the use of simple morphological stains. In tissues other than those of the oral cavity, these techniques have shown changes in connective tissue ground substance and basement membrane associated with aging, inflammation, growth, spread of tumors, and scurvy. In the oral cavity, histochemical findings in desquamative gingivitis have been described in detail.[3]

In view of the frequency in which gingival tissues are involved by inflammation, it seemed that this pathological condition should be explored using the newer histochemical techniques. A study was therefore undertaken to determine the effect of inflammation upon ground substance and basement membrane, glycogen, and alkaline phosphastase of human gingiva.

PROCEDURE

In seventeen male and female subjects, varying in age from 21 to 54, twenty-two marginal gingival biopsies were taken from areas which appeared clinically normal and areas which presented varying degrees of gingival inflammation. The biopsies thus obtained were prepared according to the Hotchkiss[2] or Lillie technique[4] for the study of ground substance, basement membrane and glycogen, and with the Gomori technique[5] for the study of alkaline phosphatase as follows:

Ground Substance, Basement Membrane, and Glycogen.—

Hotchkiss technique (Leucofuchsin periodic acid method):—The tissues were fixed for a half-hour in Carnoy's solution, dehydrated with dilutions of ethyl alcohol, cleared in xylol, and embedded in paraffin. Sections were cut at a thickness of 7 microns. Controls for free aldehydes were made for each specimen. To determine the contributing effect of glycogen in the over-all stain for ground substance, control sections were made in which the tissues were treated with fresh saliva for 15 minutes before oxidation with periodic acid. A slide of each specimen was also stained with hematoxylin and eosin for the purpose of orientation.

Supported by a grant from the Bristol-Myers Company, New York, N. Y.
Received for publication, April 24, 1951.

Modified Lillie technique:—After fixation in chilled absolute alcohol for 48 hours at 5° C., dehydration in absolute alcohol for 4 hours at room temperature, clearing in petroleum ether (using two, 30 minutes changes), and embedding for 30 minutes in paraffin at 56° C., the tissues were kept refrigerated at 5° C. until cut. Serial sections were cut at 7 microns. For the study of ground substance, the tissue was deparaffinized through the alcohol series to distilled water, placed for 10 minutes in a solution of 0.8 per cent potassium meta-periodate in 0.3 per cent nitric acid, washed in running tap water for 5 minutes, placed in Schiff's leucofuchsin reagent for 15 minutes, rinsed three times for three successive 90-second intervals in M/20 Sodium Bisulfite, washed with running tap water for 2 minutes, dehydrated to xylol, and mounted in neutral balsam.

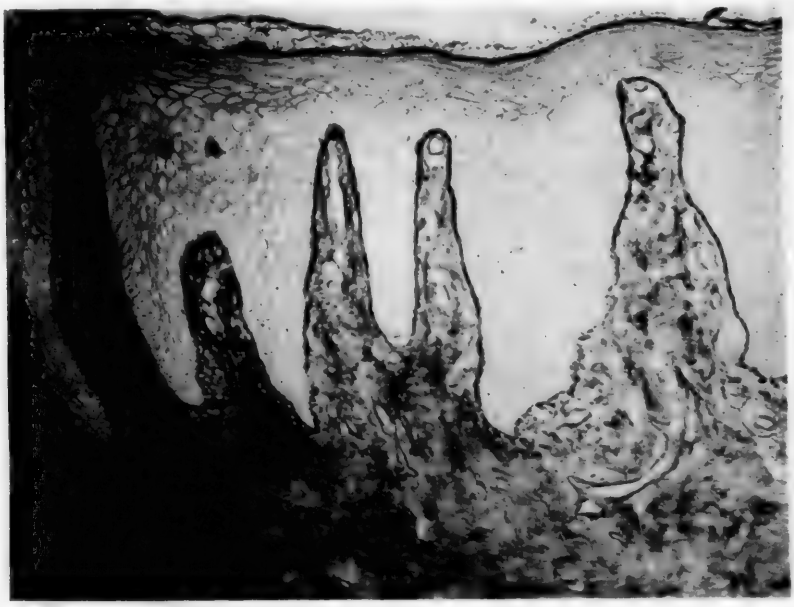


Fig. 1.—Normal gingiva showing basement membrane, ground substance and blood vessels in the connective tissue, and intercellular substance in the granular layer of the epithelium. (Lillie's periodate leucofuchsin method. Orig. mag. ×140).

For the study of glycogen the tissue was deparaffinized, incubated for 1 hour at 37° C. in diastase buffer, rinsed with distilled water before being oxidized with periodate, and then was treated as above in the modified Lillie technique. Controls for free aldehydes were conducted, substituting distilled water for the oxidizing periodate. Controls for stainable iodates were prepared by treating a section with potassium iodate instead of the oxidizing periodate.

Alkaline Phosphatase.—Each of the above techniques was supplemented with Gomori's procedure[5] to determine the presence of phosphatase. Sections treated with citrate buffer to remove preformed mineral deposits and sections processed omitting the 2-hour incubation with substrate served as controls. In some instances the sections stained for phosphatase were counterstained with 0.1 per cent safranin for 30 seconds before dehydration and mounting.

OBSERVATIONS

Normal Gingiva.—The cells of the epithelium were not stained with either the Hotchkiss or Lillie technique for ground substance, but the intercellular substance showed varying degrees of red-purple, staining fairly intensely in the hornified and granular layers of cells and becoming a mere tint in the region of the basal layer (Fig. 1). There was no concentration of glycogen in the normal epithelium. However, in occasional slides isolated scattered particles of glycogen could be seen. No alkaline phosphatase could be demonstrated in the epithelium (Fig. 2).

The basement membrane appeared as a sharp, thin line, red-purple in color, immediately beneath the basal epithelial layer. Treating the tissue with saliva or diastase did not alter the staining quality of the basement membrane.

The connective tissue presented a red-purple amorphous filmlike appearance over the entire field with an interspersed streaking of collagen fibers. The blood vessel walls stained more intensely than the surrounding field. Throughout the connective tissue it was difficult to distinguish cellular outlines.

Treating with saliva or diastase reduced the staining intensity of the connective tissue indicating the presence of glycogen in the connective tissue. The staining of the vessel walls was not altered by this treatment. Alkaline phosphatase appeared as black granules in the endothelial cells of the capillaries in the connective tissue.

Inflamed Gingiva.—The inflammation was chronic in nature with a predominance of lymphocytes and plasma cells (Fig. 3). In the epithelium overlying the inflamed connective tissue, glycogen stained with the Lillie and Hotchkiss techniques as dark purple granules concentrated in the cytoplasm of the cells of the granular and prickle cell layers (Figs. 4 and 5). Treatment with diastase or saliva reduced the staining in the epithelium indicating the removal of the glycogen (Fig. 6). Other than this difference in glycogen the epithelium overlying inflamed connective tissue stained comparably to the epithelium in normal gingiva.

In the inflamed area, the basement membrane appeared less clearly outlined, and much less intense in color than the basement membrane of normal tissue. There was no distinguishable change in the appearance of the basement membrane after treating the tissue with diastase or saliva for the removal of glycogen.

In the connective tissue of the inflamed gingiva the diffuse amorphous ground substance seen in the normal tissue was not present. Visible instead were faintly staining, delicate fibrinoid strands. Blood vessels were not so distinctly stained as in the normal gingiva. Treatment of the tissue with diastase or saliva for the removal of glycogen resulted in only a slight diminution of staining intensity, indicating a marked reduction or absence of glycogen as compared with the normal connective tissue (Figs. 5 and 6). Blood vessels appeared to be unaffected by treatment with diastase or saliva.

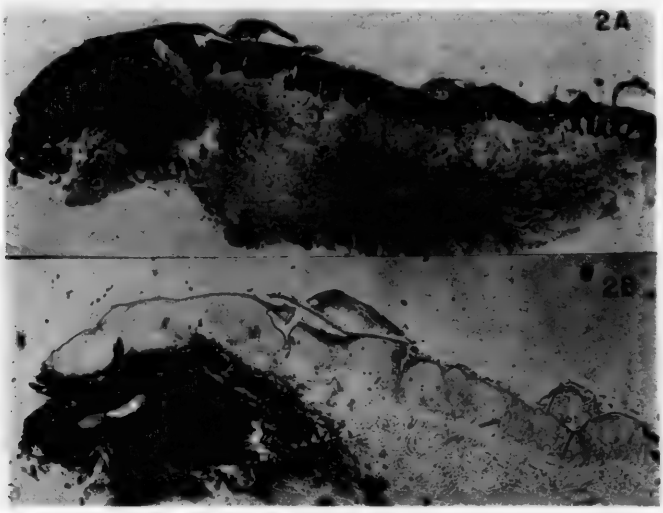


Fig. 2.—*A*, Gingival biopsy showing marginal zone of inflammation. (Hematoxylin and eosin. Orig. mag. ×15). *B*, Gingival biopsy, serial section of tissue in Fig. 2*A*, showing accumulation of alkaline phosphatase in zone of inflammation. (Gomori's technique. Orig. mag. ×15).

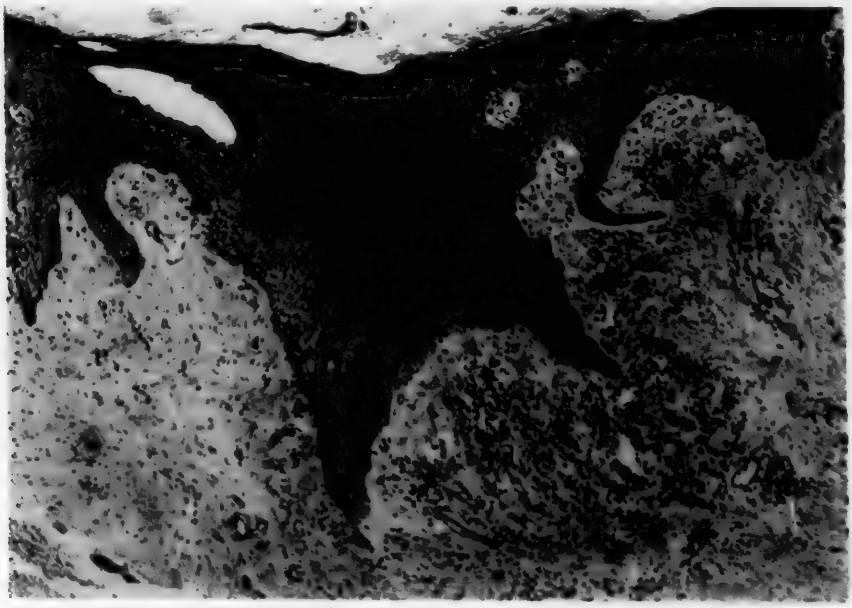


Fig. 3.—Showing proliferation of epithelium in relation to zone of chronic inflammation in the underlying connective tissue. (Hematoxylin and eosin. Orig. mag. ×130).

Alkaline phosphatase accumulated in the inflamed connective tissue (Fig. 2). It appeared in the cells of the inflammatory exudate (some of which stained more intensely than others), in the fibrocytes, the endothelial cells of the capillaries, and less notably in the collagen fibers and between the fibers.

DISCUSSION

Inasmuch as the Lillie and Hotchkiss stains as employed in this study are fairly specific for water-insoluble and alcohol-insoluble polysaccharides or polysaccharides containing protein complexes,[1] our findings pertain only to such insoluble substances. The findings indicate that glycogen and ground substance normally present in the connective tissue of the gingiva are markedly reduced in chronic gingival inflammation. In areas of inflammation, the basement membrane is thinned out and tends toward disintegration. The alkaline phosphatase content is increased.

Fig. 4.—Showing accumulation of glycogen in the cells of the granular and prickle cell layers of the hyperplastic epithelium. (Lillie's periodate leucofuchsin method. Orig. mag. ×130).

The formation and destruction of ground substance has been analyzed in detail in the literature.[1, 6, 7] It has been suggested that ground substance is formed by fibroblastic activity and broken down by depolymerizing enzymes.[1] The opinion has also been offered that the substances required by the fibroblasts for the formation of ground substance is made available by transportation from areas of the body other than the site at which it is being formed.[6]

Our findings regarding glycogen are of interest. Normally, only scattered granules of glycogen could be demonstrated in the epithelium while they appeared in greater amount and were more generally distributed in the connective

tissue. In zones of gingival inflammation, glycogen was prominent in the cytoplasm of the epithelial cells, but appeared reduced or absent in the connective tissue. In a previous histochemical study[3] glycogen was demonstrated in epithelial cells of normal gingiva, while it appeared in the connective tissue only infrequently as granules in the cytoplasm of the fibroblasts.

Fig. 5.

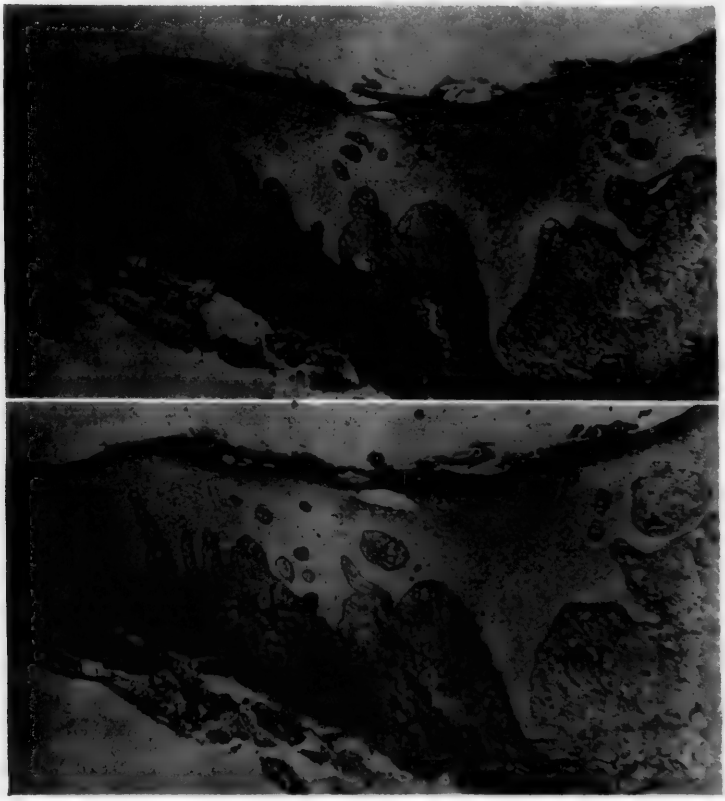


Fig. 6.

Fig. 5.—Low power survey showing difference in glycogen distribution in the epithelium overlying normal and inflamed connective tissue, and diminution of ground substance in the inflamed connective tissue (right) (Lillie's periodate leucofuchsin method. Orig. mag. ×20).

Fig. 6.—Same area as Fig. 5. Serial section showing elimination of glycogen after treating with diastase. (Lillie's periodate-leucofuchsin method. Orig. mag ×20).

The fact that glycogen did not appear in appreciable quantity in normal epithelium in this study, but was found by others,[8] may be attributed to differences in fixation. The freezing-drying technique which is the method of choice for histochemical study was used in the latter investigation, whereas we used alcoholic fixation. However, Bradfield[4] maintains that it is unlikely for glycogen which is demonstrable by freezing-drying to escape detection by alcohol fixation.

It is noteworthy that in our sections in which glycogen was prominent in the epithelium overlying areas of inflammation, it was not apparent in the epithelium of the adjacent noninflamed areas. This suggests that if the glycogen were present in the epithelium of noninflamed areas in any appreciable quantity, it would be stained by the technique used here. The possibility does exist that glycogen may be present in such small amounts in the normal gingival epithelium that alcoholic fixation techniques are not sensitive enough to pick it up.

In chronic gingival inflammation, there is an accumulation of phosphatase in the connective tissue. The phosphatase in the inflamed zone stains diffusely and is seen in individual fibrocytes and inflammatory cells in addition to its normal distribution in the endothelial cells of the blood vessels. The reason for the accumulation of phosphatase in the area of inflammation is not clear. It has also been described in the inflammatory zones of regenerating skin wounds.[7, 9] Studies of films of human blood and bone marrow preparations[10] indicate no phosphatase in lymphocytes. This suggests the possibility that the phosphatase seen in the area of inflammation and in the lymphocytes in that area may be there as a result of simple mechanical diffusion phenomena associated with circulatory alterations rather than in a specific role.

SUMMARY

Histochemical study revealed the following alterations in chronic gingivitis: diminution in connective tissue ground substance and glycogen, a tendency toward disintegration of the basement membrane and an accumulation of phosphatase in the connective tissue. In zones of chronic gingival inflammation hyperplastic epithelium presented an increased intracellular accumulation of glycogen.

REFERENCES

1. Gersh, I., and Catchpole, H. R.: The Organization of Ground Substance and Basement Membrane and Its Significance in Tissue Injury and Growth, *Am. J. Anat.* **85**: 457, 1949.
2. Hotchkiss, R. D.: A Michrochemical Reaction Resulting in the Staining of Polysaccharide Structures in Fixed Tissue Preparations, *Arch. Biochem.* **16**: 131, 1947.
3. Engel, M. B., Ray, G. G., and Orban. B.: The Pathogenesis of Desquamative Gingivitis: A Disturbance of the Connective Tissue Ground Substance, *J. D. Res.* **29**: 410, 1950.
4. Lillie, R. D.: Histopathologic Technic, Philadephia, 1948, The Blakiston Company pp. 140.
5. Gomori, G.: Michro-chemical Demonstration of Phosphatase in Tissue Sections, *Proc. Soc. Exper. Biol. & Med.* **42**: 23, 1939.
6. Klemperer, P.: The Concept of Collagen Diseases, *Am. J. Path.* **26**: 505, 1950.
7. Bunting, G., and White, R. F.: Studies of Skin Wounds in Normal and In Scorbutic Guinea Pigs, *Arch. Path.* **49**: 590, 1950.
8. Bradfield, J. R. G.: Glycogen of Vertebrate Epidermis, *Nature.* **167**: 4236, 1951.
9. Fell, H. B., and Danielli, J. F.: The Enzymes of Healing Wounds. I. The Distribution of Alkaline Phosphatase in Experimental Wounds and Burns in the Rat, *Brit. J. Exp. Path.* **24**: 196, 1943.
10. Wachstein, M.: Alkaline Phosphatase Activity in Normal and Abnormal Human Blood and Bone Marrow Cells, *J. Lab. & Clin. Med.* **31**: 1, 1946.

IN VITRO SENSITIVITY OF FUSOBACTERIA TO VARIOUS ANTIBIOTICS

ROBERT R. OMATA*

National Institute of Dental Research, National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Md.

IN THE recent past, antibiotics have been utilized in the treatment of Vincent's infection or ulcerative gingivitis. Penicillin, topically applied in the form of pastes, lozenges, or mouthwashes, was first used in the treating of infections with remarkable efficacy according to the reports by Atkinson,[1] Stewart,[11] Webster and Frey,[12] Glickman,[4] and Wihr.[13] Aureomycin and streptomycin have also been used as medicament by Goldman and Bloom[5] and Rosenthal and Winters (1950).[10]

Previous workers (Lemierre, Reilly, Morin, and Rathery,[7] Prevot and Ferly,[8] and Lahelle[6]) have shown that the fusiform bacilli are sensitive, in vitro, to penicillin. The most complete work was that of Lahelle who titrated the drug against 17 strains of Fusobacteria in glucose broth, incubated anaerobically, and found all strains to be sensitive to penicillin, in vitro. The inhibitory concentrations ranged from 0.00125 to 0.005 units per milliliter of medium. Rivett and Peterson[9] found the growth of fusiform bacilli, at pH 7.2, in a medium containing 0.75 per cent peptone and 0.25 per cent yeast extract, to be inhibited by streptolin, streptothrycin, and streptomycin in the concentrations of 0.1, 0.06 and 10.0 units per milliliter, respectively.

The purpose of this work is to present findings on the in vitro sensitivity of the Fusobacteria, known to be prevalent in the lesions of ulcerative gingivitis, to various antibiotics now available and being used in the therapy of fusospirochetal infections.

EXPERIMENTAL MATERIALS AND METHODS

Seven strains of Fusobacterium, five of which were originally isolated from acute cases of ulceromembranous gingivitis, and two of which were isolated from normal mouths (strains HA1 and HA2) were employed in this study.† The microorganisms were maintained in Brewer's fluid thioglycollate medium with a small amount of powdered calcium carbonate added and subcultures from this medium to the same medium without the $CaCO_3$ were used in preparing the inocula. It was found that the use of $CaCO_3$ in the medium assured viability of the stock cultures for a period of 7 to 10 days as against 5 to 6 days in the thioglycollate medium devoid of $CaCO_3$. The fluid thioglycollate medium, at pH 7.2, used in the drug assays, was dispensed in 16 by 150 mm. test tubes in 9 milliliter quantities and autoclaved for 15 minutes at 120° C.

Appropriate concentrations of the antibiotics were added aseptically to the cooled medium from sterile stock solutions and mixed by shaking. Stock

Received for publication, May 9, 1951.

*Fellow, Research Council, American Dental Association.

†The original stock cultures were kindly supplied by Dr. E. G. Hampp.

solutions of the antibiotics were prepared by dissolving those drugs supplied in a sterile form in sterile saline or passing saline solutions of the "nonsterile" drugs through a Seitz bacterial filter.

Each tube was inoculated with 0.1 ml. of 5 per cent saline suspension of the respective organisms. The inocula were prepared by centrifuging 48-hour cultures and resuspending in sufficient saline to give approximately 5 per cent cells by volume. The assays were read visually for growth and recorded after 4 and 7 days, in some cases again after 14 days' incubation at 37° C.

RESULTS AND DISCUSSION

The results tabulated in Table I show penicillin G to be the most effective antibiotic in the inhibition of growth of Fusobacteria, in vitro. Terramycin showed rather high activity. It will be noted that aureomycin, chloramphenicol and tyrothricin are approximately equal in activity. Fusobacteria are quite resistant to both streptomycin and dihydrostreptomycin.

It was of interest to learn whether sodium thioglycollate would have any effect on the inhibitory action of the various antibiotics. The assays were repeated using a medium similar in constitution to Brewer's thioglycollate medium except that the reducing agent, sodium thioglycollate, was omitted. The differences in the quantitative amounts were insignificant or negligible.

TABLE I

INHIBITORY CONCENTRATIONS OF ANTIBIOTICS AGAINST FUSOBACTERIA

ANTIBIOTICS	STRAINS OF FUSOBACTERIA						
	HA1	HA2	HO1	HO2	CN1	CN2	CN3
Penicillin G units per ml.	0.05	0.1	0.1	0.05	0.1	0.25	0.05
Terramycin mg. per ml.	<0.5	1.0	2.5	2.5	1.0	0.5	1.0
Chloramphenicol mg. per ml.	25	10	25	10	25	25	50
Aureomycin mg. per ml.	2.5	10	2.5	10	5	25	5
Tyrothricin mg. per ml.	>50	2.5	50	5.0	25	>50	>50
Dihydrostreptomycin mg. per ml.	500	100	250	50	100	500	250
Streptomycin mg. per ml.	1,000	100	1,000	500	250	1,000	1,000

Foley[2] tested the sensitivity of *Bacteriodes funduliformis* and *B. fragilis*, which are somewhat closely related to Fusobacterium, to penicillin and streptomycin and he found that 100 units of penicillin per milliliter of thioglycollate medium failed to inhibit growth in 48 hours. It was found that 2,500 to 4,000 units of streptomycin per milliliter of medium inhibited growth. It was also found that the presence of sodium thioglycollate did not significantly reduce the action of streptomycin on Bacteriodes.

In the course of this study stains of Fusiform bacteria growing in sublethal amounts of penicillin were examined for any changes in their morphology. Frey[3] made stained smears from gingival tissues of patients with a fusospirochetal infection undergoing treatment with penicillin. This worker reported variations in size and shape of the cells ranging from long, filamentous chains

to club-shaped and dumbbell-shaped appearances. However, in the present study on pure cultures, no morphological changes attributable to penicillin were observed.

The results obtained in this study show that penicillin G and terramycin among the antibiotics studied are the most effective in inhibiting the growth of Fusobacteria in vitro.

REFERENCES

1. Atkinson, H. F.: Observations on the Treatment of Over 100 Cases of Acute Ulcerative Gingivitis With Penicillin Pastilles, *Brit. D. J.* **79**: 317, 1945.
2. Foley, G. E.: In Vitro Resistance of the Genus Bacteriodes to Streptomycin, Science **106**: 423, 1947.
3. Frey, H.: Characteristic Morphological Changes in Bacillus Fusiformis in the Course of Penicillin Treatment, *Brit. D. J.* **82**: 135, 1947.
4. Glickman, I.: The Use of Penicillin Lozenges in the Treatment of Vincent's Infection and Other Acute Gingival Inflammation, *J. A. D. A.* **34**: 406, 1947.
5. Goldman, H. M., and Bloom, J.: Treatment of Acute Ulcerative Necrotizing Gingivitis With Aureomycin, *Oral Surg., Oral Med., & Oral Path.* **2**: 1128, 1949.
6. Lahelle, O.: Penicillin Sensitivity of Fusobacterium and Bacteriodes Funduliformis, *Acta path. et microbiol. Scandinav.* **24**: 567, 1947.
7. Lemierre, A., Reilly, J., Morin, M., and Rathery, M.: Action de la penicilline dans quelques septicemies a microbes anaerobes, *Presse Med.* **54**: 49, 1946.
8. Prevot, A. R., and Ferly, A.: Application de la methode au verte janus a la determination de la penicillio-sensibilite de anaerobes, *Bull. Acad. Med. Paris* **130**: 123, 1946.
9. Rivett, R. W., and Peterson, W. H. : Streptolin, a New Antibiotic From a Species of Streptomyces, *J. Am. Chem. Soc.* **69**: 3006, 1947.
10. Rosenthal, S. L., and Winters, G. R.: Comparative Studies of Streptomycin and Penicillin in Treatment of Infections of Oral Mucosa, *J. D. Res.* **29**: 237, 1950.
11. Stewart, G. G.: Comparative Methods of Using Penicillin in the Treatment of Vincent's Infection and Other Oral Lesions, *J. A. D. A.* **33**: 725, 1946.
12. Webster, J. F., and Frey, H.: Clinical and Microscopical Observations in the Treatment of Vincent's Infection With Penicillin, *Brit. D. J.* **82**: 70, 1947.
13. Wihr, N. L.: Penicillin in the Treatment of Vincent's Infection, *J. A. D. A.* **34**: 465, 1947.

TWO STUDIES OF PERIODONTAL DISEASE IN THE SYRIAN HAMSTER

DAVID F. MITCHELL AND D. S. CHERNAUSEK

University of Minnesota, School of Dentistry, Minneapolis 14, Minn.

PERIODONTAL disease has been observed in hamsters maintained under various conditions.[1, 2, 3] As noted in the foregoing reports, certain aspects of this disease have some points in common with its clinical counterpart in human beings.

METHODS

The data were gathered from two studies primarily concerned with dental caries.[4, 5, 6] The periodontal score, as evaluated in these studies, is based on the presence of definite large white masses of soft material in the gingival crevices of molar teeth (Fig. 1). This material appears to be associated with the finely ground, high carbohydrate diet used as the cariogenic agent. Each area involved is given the score of one unit and no attempt is made to adjudge degree of involvement. The areas involved are the buccal and lingual gingivae of the molar teeth and the mesial gingiva of the first molar and distal gingiva of the third molar. Thus, the highest score for one jaw quadrant would be 8, and for the full mouth, 32. This is a simplified adaptation of a previously described scoring method.[7] The animals of both studies were distributed according to sex and littermates at the age of 30 days, and were maintained under experimental conditions for 100 days. After sacrifice, the jaws were studied in the fresh state under a dissecting microscope.

In the "ammoniated study"[4, 5] all groups were fed a high carbohydrate caries (and periodontal disease) producing diet.* (Group 1, control; Group 2, daily brushing, control dentifrice; Group 3, daily brushing, ammoniated dentifrice; and Group 4, ingestion of ammoniated products with diet.) The periodontal scores were subjected to statistical analysis, testing the difference between mean scores.

The "sugar level study," subsequently considered, was concerned with three groups which received different amounts of confectionary sugar in their diets (Group I, 15 per cent, Group II, 30 per cent, and Group III, 45 per cent. Generally speaking, the sugar added to the diets of Groups II and III was at the expense of proportionate amounts of the first three ingredients). Mean periodontal scores also were calculated for these animals.

Supported in part by a grant-in-aid from the Graduate School, University of Minnesota.
Received for publication, June 1, 1951.

*Whole wheat flour, 30 per cent; whole powdered milk, 30 per cent; corn starch, 20 per cent; confectioner's sugar, 15 per cent; alfalfa meal, 4 per cent; and sodium chloride, 1 per cent.

RESULTS OF "AMMONIATED" STUDY

Table I does not show any significant difference in scores, at the .01 level of probability, that could be attributed to a sex factor; however, the females of all groups had a slightly higher mean value in all comparisons. It is believed by most investigators that male hamsters develop more caries than the females.

TABLE I

DIFFERENCE BETWEEN SEXES

GROUPS TESTED	NO. ANIMALS	MEAN	STANDARD DEVIATION	t	p
♀ All	52	6.63	4.87		
♂	51	5.22	3.84	1.61	.120
♀ G1	14	8.14	4.69		
♂ G1	14	5.43	4.71	1.46	.146
♀ G2	13	8.77	4.04		
♂ G2	14	6.57	3.54	1.45	.146
♀ G3	12	3.92	3.40		
♂ G3	11	3.55	2.78	.27	>.656
♀ G4	13	5.38	5.37		
♂ G4	12	4.92	3.00	.25	>.656

t The ratio of any normally distributed statistic to its standard error of estimate.
p Probability.

Table II shows the comparison of periodontal scores among the different groups. The male and female scores are combined since the results shown in Table I did not reveal a marked sex difference. It is of interest to note that the ammoniated brushing (group 3) had the lowest score. A significant difference at or below the .01 level of probability was found only when group 3 was compared with group 2. No reduction of periodontal involvement due to brushing, per se (group 2), was shown.

TABLE II

DIFFERENCE BETWEEN GROUPS

GROUPS TESTED	N	$\bar{x}$	S_x		t VALUES / PROBABILITY 1	2
1	28	6.79	4.84	1	—	—
2	27	7.63	3.95	2	−.69 .489	—
3	23	3.74	3.12	3	2.53 .018	3.74 .001
4	25	5.16	4.41	4	1.23 .240	—

Since the "ammoniated" groups (3 and 4) had the lowest periodontal scores, their data were compared with those of the "nonammoniated" groups (1 and 2). It was found that such a difference between the means of these groups should occur by chance only 3 times in one thousand (mean scores 4.48, 7.20 and standard deviations 4.50, 3.91 respectively).

RESULTS OF "SUGAR LEVEL" STUDY

Table III reveals the data for the three groups fed increasingly larger amounts of sugar in the diet. A greater number of animals was used. Again, the female scores are slightly higher than the males, but the difference is not great. No importance is attached to the differences existing among mean scores of the three groups. The scores from this study were considerably lower than those obtained from the "ammoniated" study.

TABLE III
DIET STUDY

	N	$\bar{X}$
Group I		
♀	28	2.57
♂	27	1.48
♀ ♂	55	2.04
Group II		
♀	27	2.07
♂	28	1.96
♀ ♂	55	2.02
Group III		
♀	26	1.92
♂	28	1.57
♀ ♂	54	1.74

DISCUSSION

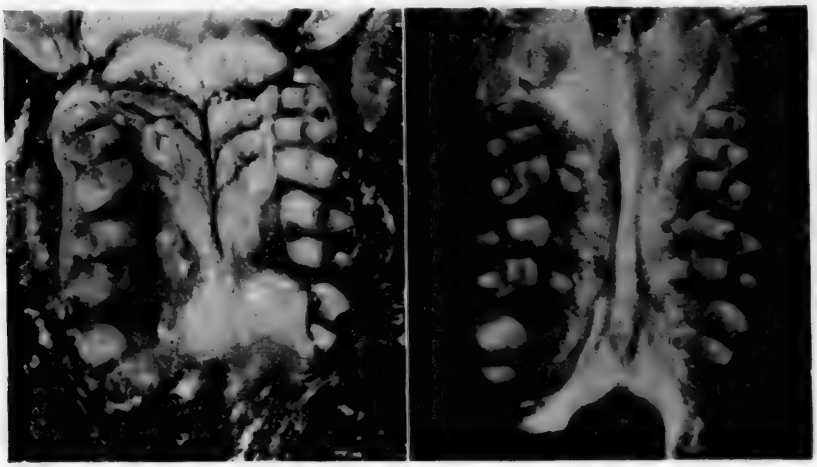
The mechanism of development of periodontal disease in the hamster is not clearly defined. The gingival accumulations have been noted in association with soft, high carbohydrate diets and with periodontal destruction (Fig. 1) by several investigators.

King has called this material "calculus" and felt that it was most heavily deposited in the regions of the openings of salivary ducts.[8] Others have considered the material to be "plaque,"[1] "bacterial deposit,"[9] or other forms of soft accumulations unrelated to the position of salivary ducts.

Concerning the present report, an effort was made to use animals of littermate distribution in the two studies. The diet for the first group of each study was the same. Experimental periods and maintenance conditions were the same, although the studies were conducted several months apart. There was a disparity between the mean scores of the two studies which we cannot explain: e.g., the combined mean scores of the males and females of group 1 of the "ammoniated" study was 6.79, and the similar figure for the identically treated group I of the "sugar level" study was 2.04. Nevertheless, we believe that the scoring method will detect differences among groups in any one experiment.

Those animals receiving ammoniated products by tooth brushing or ingestion (groups 3 and 4) had less gingival accumulations. These accumulations have been shown to be intimately associated with a form of root cavitation.[7]

We believe the accumulations to be organic in nature, somewhat similar to the caries plaque. Why the ammoniated products inhibited this gingival plaque formation is a matter for speculation.



A. *B.*

Fig. 1.—*A*, The gingival debris and soft tissues are shown in situ. *B*, The same jaw stripped and cleaned. Note bone loss and displacement of molars, and resulting diastemata.

CONCLUSIONS

There is need for further study of the relationship between periodontal disease and caries in the hamster. The scoring method proved to be definitive enough to detect differences between groups. Although a trend toward higher periodontal disease scores in the females was shown, no clear-cut sex difference was established. Ammoniated products were associated with a reduced amount of periodontal involvement. No appreciable difference in the amount of periodontal disease was noted among the groups which received 15, 30, and 45 per cent confectionary sugar in the diet.

REFERENCES

1. Keyes, P. H., and Likins, R. C.: Plaque Formation, Periodontal Disease and Dental Caries in the Syrian Hamster, *J. D. Res.* **25**: 166, 1946.
2. Keyes, P. H.: Dental Caries in the Syrian Hamster, *J. D. Res.* **25**: 34, 1946.
3. Mitchell, D. F.: Introductory Observations of the Periodontium of the Syrian Hamster, *J. D. Res.* **27**: 330, 1947.
4. Chernausek, D. S., and Mitchell, D. F.: Ammoniated Dentifrices and Hamster Caries, Part I, The Effect of Ingestion, *Science* **112**: 273, 1950.
5. Chernausek, D. S., and Mitchell, D. F.: Part II. The Effectiveness of Brushing the Teeth With a Control and an Ammoniated Dentifrice. To be published.
6. Mitchell, D. F., Chernausek, D. S., and Helman, E. Z., Hamster Caries. The Effects of Three Sugar Levels in the Diet, and an Evaluation of Scoring Procedures. To be published.
7. Mitchell, D. F.: The Production of Periodontal Disease in the Hamster as Related to Diet, Coprophagy, and Maintenance Factors, *J. D. Res.* **29**: 732, 1950.
8. King, J. D.: Histological Observations on Parodontal Disease in the Golden Hamster (Cricetus Auratus): Calculus, Food Particles, and Other Foreign Bodies as Aetiological Factors, *J. Path. & Bact.* **61**: 413, 1949.
9. Rushton, M. R.: The Epithelial Downgrowth on the Molar Roots of Golden Hamsters, *Brit. D. J.* **90**: 87, 1951.

DENTAL DISEASE IN PSYCHIATRIC PATIENTS

JAMES M. DUNNING, ROBERT W. HYDE, AND PETER J. DALTON

Harvard School of Dental Medicine and Boston Psychopathic Hospital, Boston, Mass.

ALTHOUGH much has been written on the psychosomatic aspects of dental treatment, very little attention has been paid to the influence of psychic disturbance upon the production of dental disease. Such material as is available deals chiefly with soft tissue disturbances.[1] Dental caries has not been studied in any systematic fashion, although alterations in physiology have been noted in certain types of mental disease of a nature to suggest changes in caries incidence. For example, schizophrenics have been observed to show an increased rate of salivation[2] and salivary pH has been seen to rise under temporary emotional stress.[3]

In an effort to study dental caries experience among psychiatric patients, dental examinations in terms of DMF teeth (decayed, missing and filled) were recorded over a period of almost three years on as many patients as possible admitted to the Boston Psychopathic Hospital. Approximately 95 per cent of all patients admitted received these examinations in the office of the staff dentist (P.J.D.) and the only patients omitted were those who were too disturbed during their entire stay in the hospital. Those patients who were at first too disturbed to report to the dentist's office but who later improved, received dental examination as soon as improvement took place. The average length of stay in the Boston Psychopathic Hospital is nine weeks. The hospital is a diagnostic, intensive treatment, research and training center so that it has a high proportion of patients who have had a short duration of mental illness. For this reason our data are not necessarily comparable to what might be found in hospitals largely caring for chronic or long-term mental diseases. However, there are many "repeaters" who have had recurrent attacks, several transfers from state hospitals for special treatment, and some patients who have been mentally ill for a considerable time but hospitalization had been delayed. Further study upon chronic mental patients is indicated.

The usual dental examination at the Boston Psychopathic Hospital is performed in a dental chair with adequate light, mirror, and explorer. The whole mouth is examined and a record made of all carious, missing, and filled teeth. Bridges and artifical dentures are examined and records made of them. Note is also made of extensive caries and teeth too broken down for filling. Third molar conditions are recorded, but have been omitted from the analyses which follow because of lack of x-ray evidence which would permit differentiation between extracted and unerupted or impacted teeth. The average examination takes about five minutes. One dentist has performed the entire series. The

Read at a meeting of the Boston Section International Association for Dental Research, June 7, 1951.

Received for publication, April 7, 1951.

diagnoses of mental disease have been made by various members of the medical staff of the hospital and often are the result of staff conference. They have been categorized according to the code used by the Department of Mental Diseases of the Commonwealth of Massachusetts. Schizophrenia, being synonymous with dementia praecox, has been recorded under the latter heading.

One of the first problems involved in the analysis of the data lay in the finding of a good base line with which the individual mental diagnostic groups could be compared. The wide differences in caries experience figures which may result from different methods of examination used by different operators and under different circumstances are well known.[4] For this reason, and because no outside dental data were available in the area compiled in a manner closely similar to that at the Boston Psychopathic Hospital, we were reluctant to rely upon outside comparisons. On the other hand, there was a danger that the entire patient population of the Hospital might differ from any comparable population without mental disease. This latter danger is perhaps the lesser of the two, since a very wide range of mental states including many quite minor disturbances is found in the patient group. We have therefore taken the hospital group as our baseline, comparing dental findings for any one mental diagnosis with the dental data for all cases.

Comparison of the hospital data with the best available outside groups is made in Table I. It will be seen that the mental patients fell below the industrial employees studied in New York in 1942[5] and the military selectees studied in Boston in 1943[6] as to dental caries experience. This result may be due entirely to examination technique. The industrial employees were examined by dental hygienists supervised by dentists and with the benefit of frequent dental x-ray surveys. The examinations were repeated every six months and cumulative findings are often more reliable and complete than those from any one examination. Hence the difference between the industrial employees and the mental patients has probably been exaggerated. The military selectees on the other hand were examined under hurried conditions, and caries recording may be incomplete. To offset this, missing third molars are apparently included in the DMF counts. As a result of these comparisons, it seems unlikely that important differences in dental caries experience exist between the patient population at Boston Psychopathic Hospital and other easily available adult groups.

Table II shows the age composition of the entire group of psychopathic patients as well as DMF rates for those between 10 and 50 years of age. It will be noted that there were a few patients under the age of 10, too small a group to justify analysis. Above age 50 a much larger group was found, but again the number of patients within any one five-year age group was relatively small and the DMF rates so very scattered as to throw serious doubt upon any inferences to be drawn from them. Moreover, by confining the DMF data to patients under fifty, attention is concentrated on dental caries. Above the age of fifty so many teeth are lost through periodontal disturbances that the "missing" component in the DMF rate no longer renders a reliable picture of the effects

of dental caries. Even in the 45- to 49-year age group, rough figuring, based on experience elsewhere[7], indicates that about 30 per cent of the missing tooth count (M) may be due to tooth loss from periodontal disease.

TABLE I

DMF TEETH IN CERTAIN ADULT POPULATION GROUPS

PSYCHOPATHIC PATIENTS 1946-1948*		INDUSTRIAL EMPLOYEES NEW YORK, 1942*†		MILITARY SELECTEES, BOSTON, 1943‡		
AGE GROUP	DMF TEETH PER PERSON	CASES	DMF TEETH PER PERSON	AGE	CASES	DMF TEETH PER PERSON
10-14	6.58					
15-19§	8.32	200	12.93	18	1205	11.0
20-24	10.83	200	15.44	22	706	12.5
25-29	11.38	200	16.44	26	1109	14.0
30-34	14.57	200	16.90	30	1116	15.0
35-39	15.38	200	17.91	34	929	16.5
40-44	17.16	200	18.28	37	‖	18.0
45-49	18.88	200	20.12			

*Data exclude third molars.

†Dunning, J. M. and Klein, H. Saving teeth among Home Office Employees of the Metropolitan Life Insurance Co., *J.A.D.A.* **31**: 1634, 1944.

‡Hyde, R. W., unpublished data from Boston Induction Center.

§Ages 17-19 for the industrial employees.

Analyzing the DMF rates found in the second part of Table II and in Fig. 1, the impression one receives is that no one diagnostic group differs very sharply throughout the entire age span from the average for all cases. Minor differences do appear in several instances. These are worthy of statistical analysis. The DMF rates for patients with manic-depressive psychosis are above the average in every age group. The DMF rates for patients with primary behavior disorders and psychopathic personality without psychosis (these two diagnoses are considered to represent a continuum) show many more differences below the average than they do above the average. DMF rates for patients with alcoholism (without psychosis) are sharply above the average in the age groups 30 through 44 although the entire series of differences from age 20 through age 49 shows no very striking variation from the average. The DMF rates for patients with dementia praecox are evenly distributed above and below the average for all cases, but the deviations below the average are the greater ones. All of these differences confirm clinical impressions current among the staff at the Boston Psychopathic Hospital.

Table III presents the differences in DMF teeth, at each age group, between the mean for all cases and the mean for each of the diagnostic groups. The value for all cases is taken to be zero and the difference is given a sign in accordance. The right hand columns of each pair in Table III are critical ratios obtained by the analysis of frequency distributions for each age-diagnostic group.* Critical ratios, it will be remembered, indicate the probability that a

*The formula used was $C.R. = \frac{\Delta}{\sigma_{obs}\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$

where Δ is the difference between means, σ_{obs} the standard deviation of the observations in the "all cases" group and n_1 and n_2 the number of cases respectively in each of the two samples undergoing comparison.

TABLE II

FREQUENCES OF DIFFERENT MENTAL DIAGNOSES AND CORRESPONDING MEAN NUMBERS OF DMF TEETH AMONG IN-PATIENTS RECEIVING DENTAL EXAMINATION AT BOSTON PSYCHOPATHIC HOSPITAL 1946-48, TABULATED ACCORDING TO AGE AT LAST BIRTHDAY

	FREQUENCY OF CASES												DMF TEETH PER PERSON*							
DIAGNOSIS	ALL AGES	UNDER 10	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50	UNKNOWN	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49
All cases†	2692	3	88	294	332	357	327	288	247	279	460	17	6.58	8.32	10.83	11.38	14.57	15.38	17.16	18.88
Primary behavior disorders	144	2	39	70	7	7	5	4	1	2	6	1	5.70	6.99	10.71	12.14				
Without psychosis (all types)‡	833	1	28	121	142	126	102	83	72	80	121	7	6.82	8.30	10.79	11.23	14.93	15.60	18.00	17.95
Psychopathic personality without psychosis	362	0	12	67	86	65	40	28	19	17	24	4	7.16	8.42	10.41	11.28	12.50	13.78	19.88	16.70
Alcoholism without psychosis	225	0	0	3	14	28	38	38	30	38	35	1			11.43	10.74	15.97	17.31	17.82	18.25
Dementia Praecox (all types)‡	536	0	6	55	83	106	103	67	47	29	37	3	5.67	9.37	9.98	11.17	14.70	14.57	14.10	19.47
Paranoid type, dementia praecox	234	0	0	13	32	43	42	36	26	19	22	1		11.12	11.49	15.90	13.90	14.42		
Manic depressive psychosis§	187	0	1	13	21	24	28	25	19	28	26	2		10.93	12.00	11.83	15.53	16.27	19.20	19.35
Psychosis with syphilitic meningoencephalitis	165	0	1	1	0	7	6	25	30	39	55	1						18.55	17.87	18.95
Alcoholic psychosis	135	0	0	0	6	6	22	26	24	16	35	0					14.04	14.00	17.00	
Psychoneurosis	213	0	4	22	31	40	39	23	14	24	15	1		9.14	11.28	11.82	12.39	14.60		21.08

*Samples of under 20 cases are averaged only when part of a series of particular interest.

†Includes subgroups that do not justify dental analysis, as follows: involutional psychosis 127 (most are over 50 years of age), paranoia and paranoid conditions 64, undiagnosed psychosis 85.

‡Includes other subgroups than those analyzed.

§Includes 78 manic type cases, 78 depressive type, and 31 other types.

given difference between two mean values might have occurred by chance. A critical ratio of 2.00 indicates approximately a 5 per cent probability of chance occurrence and a critical ratio of 3.00 a probability of approximately 0.3 per cent. The critical ratios of Table III have been given signs corresponding with the differences they test. Fig. 2 shows the critical ratios in graphic form.

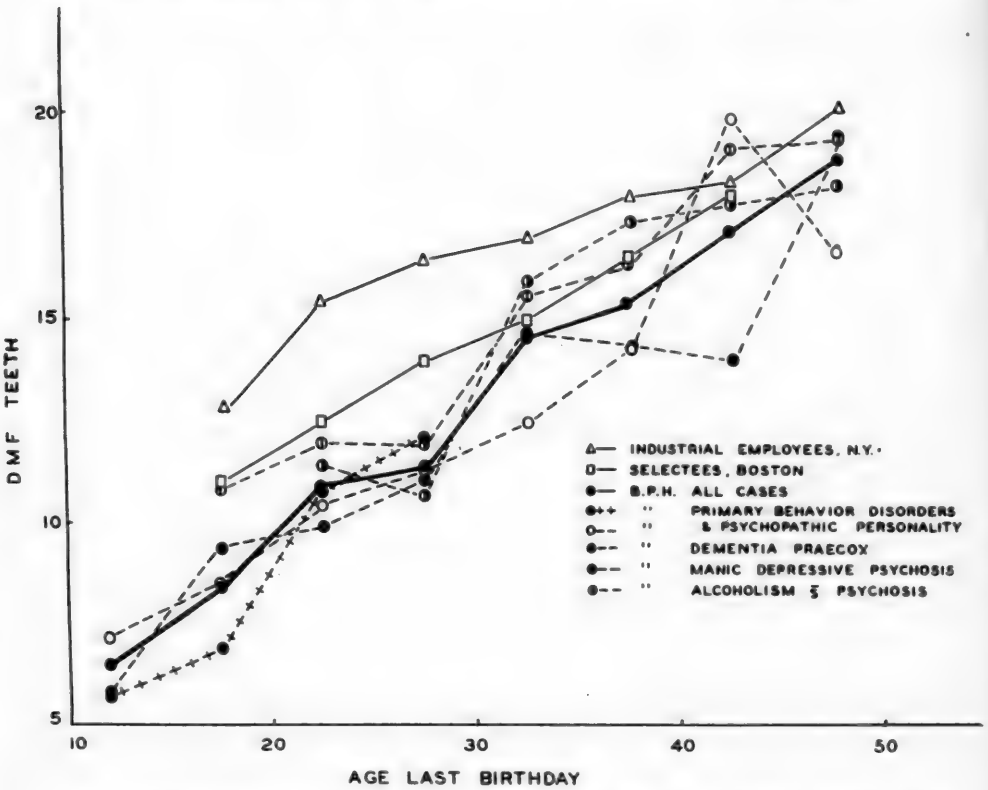


Fig. 1.—Mean numbers of D.M.F. teeth per person in certain mental diagnostic groups, Boston Psychopathic Hospital 1946-1948 and in certain other adult groups.

It is noteworthy how few of these ratios approach even the 5 per cent level in significance (2.00). This is in part because five-year age groups have been selected with the result that samples are small. Larger samples, as for instance 10-year age groups, would probably show larger critical ratios but might introduce error because of the weighting of the two samples in any given pair at opposite ends of the age span.

It is interesting to see what happens when larger age groups are taken in those instances where it is possible to do so without undue weighting of the samples. Table IV shows how well a 20-year sample of patients with manic depressive psychosis compared in percentage age composition with the similar 20-year sample for all cases. This sample shows an average DMF rate of 1.10 teeth

TABLE III

DIFFERENCES IN DMF TEETH BETWEEN SAMPLES OF ALL CASES AT GIVEN AGES AND SAMPLES FOR CERTAIN MENTAL DIAGNOSES AT SIMILAR AGES, BOSTON PSYCHOPATHIC HOSPITAL, 1946-1948

AGE GROUP	PRIMARY BEHAVIOR DISORDERS*		PSYCHOPATHIC PERSONALITY WITHOUT PSYCHOSIS*		DEMENTIA PRAECOX†		MANIC DEPRESSIVE PSYCHOSIS†		ALCOHOLISM WITHOUT PSYCHOSIS	
	DIFF.‡	C.R.§	DIFF.‡	C.R.§	DIFF.‡	C.R.§	DIFF.‡	C.R.§	DIFF.‡	C.R.§
10-14	−.88	−1.13	+.58	+0.15	−.91	−0.53				
15-19	−1.33	−2.22	+.10	+0.16	+1.05	+1.58	+2.61	+2.04		
20-24	−.12	−0.06	−.43	−0.66	−.85	−1.29	+1.17	+0.97	+.60	+0.41
25-29	+.76	+0.38	−.11	−0.15	−.21	−0.36	+.45	+0.40	−.64	−0.62
30-34			−2.07	−2.24	+.13	+0.21	+.96	+0.88	+1.40	+1.48
35-39			−1.60	−1.22	+.81	+0.90	+.89	+0.64	+1.93	+1.68
40-44			+1.72	+1.04	−3.06	−2.78	+2.04	+1.24	+.66	+0.49
45-49			−2.18	−1.24	+.59	+0.43	+.47	+0.34	−.63	−0.52
Mean critical ratio, all ages	−.60 Borderline significance				−.20 not sig.		+.93 sig.		+.49 not sig.	

*These two diagnoses are considered to represent a continuum.

†All types.

‡Difference between mean in DMF teeth for all cases (=0) and mean for specific diagnostic group.

§Critical ratio, arbitrarily given a sign to correspond with the difference.

higher than the average for all cases. The critical ratio applicable to this difference is 1.57, a much larger ratio than that shown for most of the differences between five-year samples. Table IV also shows the similarity in percentage age composition between a 15-year sample of cases of alcoholism (without psychosis) and the similar age sample for all cases, The alcoholism group showed an average DMF rate of 1.39 teeth more than the rate for the sample of all cases. The critical ratio applicable to this difference is 2.11: a ratio which indicates less than 5 per cent probability of chance occurrence and is therefore of borderline significance.

TABLE IV

AGE COMPOSITION OF EXPANDED SAMPLES FOR CERTAIN MENTAL DIAGNOSES COMPARED WITH SIMILAR SAMPLES OF ALL CASES, BOSTON PSYCHOPATHIC HOSPITAL, 1946-1948

	AGE IN YEARS TO LAST BIRTHDAY									
	30-34		35-39		40-44		45-49		ALL AGES	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
All cases	327	29	288	25	247	22	279	24	1141	100
Manic depressive psychosis	28	28	25	25	19	19	28	28	100	100
All cases	327	38	288	33	247	29	—	—	862	100
Alcoholism without psychosis	38	36	38	36	30	28	—	—	106	100

In the mean critical ratios for the entire age spans shown in Table III are to be assessed for significance, it is possible to treat them as a series of original observations.* The hypothesis to be tested is that no differences between the original DMF means are expected (null hypothesis) and that therefore the mean of the series of critical ratios should also be zero. Under these circumstances the expected standard deviation of the series of critical ratios will be 1.0.

*The following formula was used:

$$\text{Critical ratio for series of critical ratios} = \frac{\text{observed mean for series} - \text{expected for mean series}}{\sqrt{\frac{(\text{expected } \sigma \text{ series})^2}{\text{number in series}}}}$$

Under this method of figuring the drop in dental caries for the primary behavior disorder-psychopathic personality continuum is of borderline significance ($C.R._{c.r.'s} = 2.08$). The excess of low figures over high figures in the dementia praecox series turns out to be not significant ($C.R._{c.r.'s} = 0.49$). The slight rise in caries at all ages in the manic-depressive psychosis series appears to be just significant ($C.R._{c.r.'s} = 2.45$). The entire series of cases of alcoholism without psychosis shows a caries rise which is not ignificant ($C.R._{c.r.'s} = 1.20$).

The various components of the DMF rate depend so much upon past dental care that for the most part these components were considered not to be worthy of analysis in a study attempting to relate mental state with the occurrence of dental disease. In one group of patients, however, the factor of dental neglect was thought to be an important one. Older alcoholic patients (without psychosis) seemed in general a group that had gone for long periods without adequate

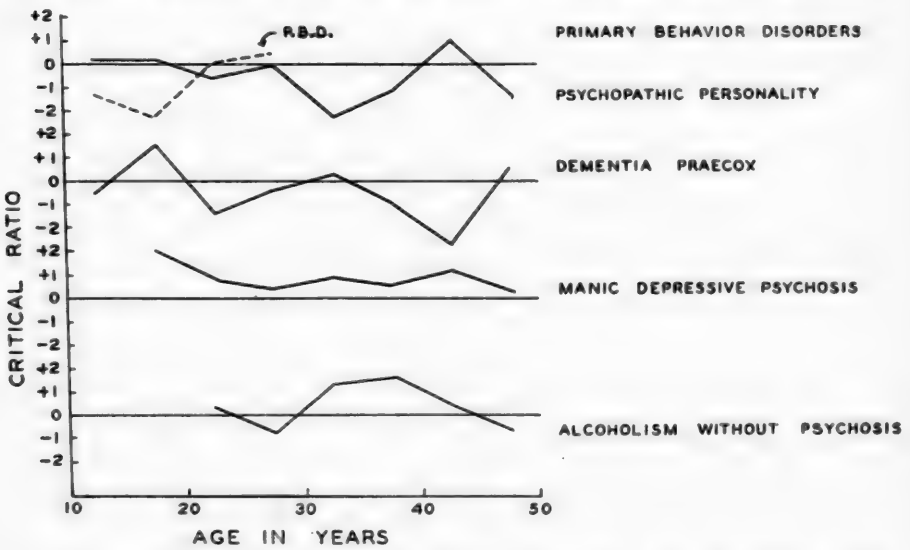


Fig. 2.—Critical ratios for differences between mean D.M.F. teeth per person in certain mental diagnostic groups and corresponding means for all cases, Boston Psychopathic Hospital, 1946-1948.

dental care and might have an unusually high number of missing teeth. Missing teeth were therefore tabulated for the age groups 30-34 and 35-39 and the mean values compared with the mean values for missing teeth among the "all cases" groups at the same ages. More missing teeth were found in both alcoholic groups than in the similar control groups. In the age group 30-34 there was an average of 2.81 more missing teeth in the alcoholic group, a difference which carries a critical ratio of 2.15. In the age group 35-39 the alcoholics had 2.11 more missing teeth per person and this difference carried a critical ratio of 1.42. Where the two groups were combined the increased sample size increased the reliability of the results. A difference of 2.52 missing teeth per person was found which carried a critical ratio of 2.54 and was therefore at least of borderline significance.

DISCUSSION

Short term mental disturbances have for a long time been thought to influence the occurrence of dental caries. One of the chief difficulties in testing this hypothesis has been that the incidence of new lesions of dental caries has been very difficult to measure over a short period of time unless exact examinations were performed immediately before and after the episode of disturbance and in other periods as well in order to include periods of normal health. The intensity of the mental disturbance ought also to be measured. The difficulty in either predicting or measuring such a disturbance is obvious. Studies of longer term psychic deviations and their relationships to dental caries are therefore of interest not only in themselves but because of the light they may throw upon the effect of shorter term deviations.

Clinicians in the field of psychiatry have come to associate certain general mouth conditions with certain mental states. The situation in manic depressive psychosis is an example. Our finding of a higher caries rate parellels clinical impression and suggests that if much larger samples had been available more significant differences from the "normal" might have been obtained. If the increase in caries is confirmed in subsequent studies, it will be of interest to search for some physiological reason. Economic and social factors may have their influence upon the occurrence of dental disease. Thus the rise in DMF teeth among alcoholics above age 30 and the accompanying rise of missing teeth have very large socioeconomic implications.

The present study is of interest almost as much for the differences which were not found as for those which were found. Many diagnostic groups show no appreciable deviation from the normal. It should be reemphasized that the study deals with relatively small numbers of patients and can at best be considered suggestive. Its greatest usefulness may be in helping to give better focus to future studies dealing with larger patient groups or cases of more chronic mental illness.

SUMMARY

A group of 2,212 in-patients at the Boston Psychopathic Hospital between the ages of 10 and 50 years were studied for possible relationships between mental state and dental disease, the latter expressed in terms of DMF teeth. This patient group as a whole shows a somewhat lower DMF rate than was found by Hyde among Boston military selectees in 1943 and a considerably lower DMF rate than was found by Dunning and Klein among industrial employees in New York City in 1942. Since these differences may be regional or due to differences in examination methods and since the hospital group showed a wide range of mental states, it was decided to use this group as its own base line. For purposes of analysis the sample was divided into 5-year age groups. Few mental diagnostic groups showed any significant difference from the DMF rates for the sample as a whole. Those differences that were found were small.

Patients with manic-depressive psychosis had a higher DMF rate at every age studied, although the differences were so small and the number of cases in the samples were so small that statistical significance was only obtained by a

small margin when the series was considered as a whole. Patients 30 to 40 years of age showing alcoholism (without psychosis) also had a higher DMF rate accompanied by a higher tooth mortality (M teeth).

Further study of larger and more chronic groups is desirable.

Grateful acknowledgment is due to Jane Worcester, Ph.D., Associate Professor of Biostatistics at the Harvard School of Public Health, for assistance in statistical method.

REFERENCES

1. Miller, S. C., and Firestone, J. M.: Psychosomatic Factors in the Etiology of Periodontal Disease, *Am. J. Orthodont. & Oral Surg.* **33**: 675, 1947.
2. Burstone, M. S.: The Psychosomatic Aspects of Dental Problems, *J. A. D. A.* **33**: 862, 1946.
3. Hyams, I. B.: Personality Factors and Dental Caries, *J. Canad. D. A.* **14**: 473, 1948.
4. Dunning, J. M.: Variability in Dental Caries Experience and Its Implication Upon Sample Size, *J. D. Res.* **29**: 541, 1950.
5. Dunning, J. M., and Klein, H.: Saving Teeth Among Home Office Employees of the Metropolitan Life Insurance Co., *J. A. D. A.* **31**: 1632, 1944.
6. Hyde, R. W.: Unpublished data assembled in connection with article: Socio-Economic Aspects of Dental Caries, *New England J. Med.* **230**: 506, 1944.
7. Brekhus, P. J.: Dental Disease and Its Relation to the Loss of Teeth, *J. A. D. A.* **16**: 2237, 1929.

THE DENTITION AS A CRITERION OF RACE WITH SPECIAL REFERENCE TO THE ALEUT

COENRAAD F. A. MOORREES, TANDARTS, D.D.S.

Forsyth Dental Infirmary for Children, Boston, Mass.

THE dentition offers a large number of characteristics in the form, size, cusps numbers and figure patterns of the teeth themselves, in pigmentation of gingiva, and hyperostosis of the jaws.[1] These variables depend in their ultimate form or expression largely on a particular assortment of genes. Therefore, we may expect group differences in the dentition of man and should determine the existence of certain combinations of specific characteristics in different populations. These criteria can then be applied to identification not only of the living, but even of skeletal remains, since teeth may be the only traces found and generally are well preserved. Consequently, present and past populations can be included in a single investigation.

Time required for the dental study in the field is relatively short. Impressions and casts are obtained rather simply and the analysis can be postponed to the laboratory. Here measurements and observations on the multitude of morphological features are made, preferably with the assistance of a co-observer.

Gingival pigmentation and palpation of the mandibular torus have to be recorded on the spot. Observations on developmental defects, color and necrotic lesions of the teeth, together with examination of the soft tissues and saliva, may be added to the study of a population in order to develop a better understanding of the problem each presents.

The purpose of the dental study in man is: (1) to add to the body of data concerning the dentition, (2) to establish possible connections in time and space between populations, (3) to differentiate within populations, (4) to study the influence of admixture, (5) to determine the mode of inheritance of specific features.

Some of these points can be illustrated by data on the Aleuts now living in the Aleutian islands of Atka and Nikolski, gathered during the Aleutian Expedition* from Peabody Museum, Harvard University, in 1948.

The first item is the so-called torus mandibularis (Fig. 1), an exostosis located on the lingual side of the mandible near the roots of canines and premolars, yet above the mylo-hyoid line. The anteroposterior limits vary with its size, but extend usually from canine to second premolar.

Many investigators have been puzzled as to its causation and possible value as a racial criterion. The torus has been attributed variously to pathologic changes, arctic conditions of life, masticatory stress, and heredity. In a review of the matter as late as 1940, Hrdlicka[2] concluded as follows: "It seems impossible to reach any other conclusion than that they are caused by stress of

From a paper read at the annual meeting of the American Society of Physical Anthropologists, Boston, Mass., March, 1950.

Received for publication, April 7, 1951.

*Supported by grants from the office of Naval Research, administered through the Pacific Science Board of the National Research Council and the Viking Fund.

mastication in excess of the capacity of the individual bones, and that they are effects of the organism to provide additional strengthening to the parts affected.'' This conclusion was in part influenced by the belief that torus was hardly ever found in the young and seemingly appears in late adolescence.

Fig. 1.—Torus mandibularis, a bony exostosis found on the lingual side of the mandible. (Specimen courtesy Peabody Museum, Harvard University).

However, the findings on the living Aleuts suggest different conclusions. Torus mandibularis was found in 35 per cent of the population; 30 per cent of children under 10 years of age exhibited ''slight'' or ''trace'' torus forma-

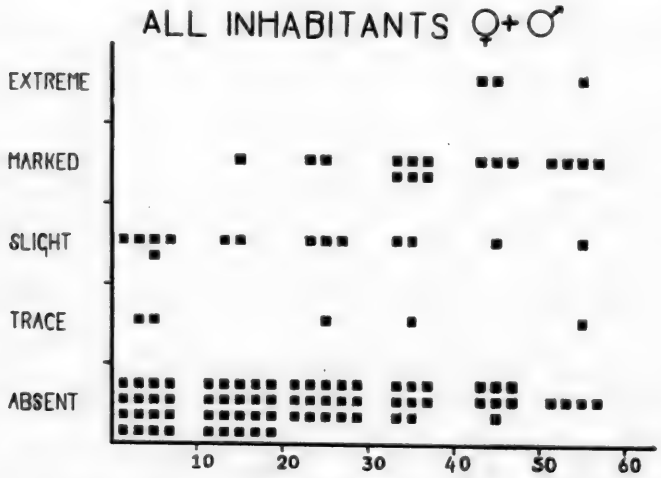


Fig. 2.—The degree of torus mandibularis formation at different age levels in the population of the Aleutian Islands.

Four stages of development of lower molar teeth, illustrating the different changes from the Dryopithecus to the most advanced pattern. *A*, Dryopithecus pattern indicated by Y_5 meaning primitive system of grooves and cusp formula. *B*, Modified Dryopithecus pattern indicated by Y_4 meaning cusp formula reduced to four and groove system primitive with loss of the posterior limb of the Y. *C*, Primitive cusp formula retained but groove system changed to cruciform, the sign being +5. *D*, Cusp formula and groove system changed, sign +4.

tion, while many older Aleuts did not have a torus. For example, a 2.36-year-old boy had a "slight" torus formation. The difference in findings in this and other studies may lie in the fact that the other studies have been done on skeletal material, where the young are very sparingly represented. Even in the Aleut, however, the suggestion is present that most marked forms appear in the older age group, as can be seen from Fig. 2.

The subjective method of appraisal through palpation can in part be responsible for inaccuracy in grading the size of torus in proportion to jaw size. Consequently, the torus of a child may sometimes be underrated as the size of the torus itself is judged rather than its size relative to the jaw. The effect of function, the geographical distribution, white admixture, and the hereditary aspects will be mentioned briefly.

In order to observe the effect of masticatory stress, a possible association between wear of the teeth as a measure of function, and the size of torus was tested, but proved to be absent. Therefore, it is assumed that torus formation is not related to stress. Additional evidence can be found in the fact that teeth of Aleut women have more wear, since they use their masticatory apparatus more for chewing sealhides, but have less torus formation. This observation is in accord with findings of other investigators.

TABLE I. DEVIATIONS OF MESIO-DISTAL TOOTH DIAMETERS IN POPULATIONS BELONGING TO MONGOLOID STOCK COMPARED TO SWEDES* (WHITES)

MAXILLARY TEETH OF:	I_1	I_2	C	PM_1	PM_2	M_1	M_2	M_3
Aleuts	−.43	+.48	−.03	− .03	− .28	− .35	− .31	− .18
Japanese	−.23	+.28	−.21	+ .19	− .11	− .39	−2.34	− .58
Chinese	−.05	+.26	+.15	+ .10	− .05	− .57	− .88	− .68
Javanese*	−.22	+.21	+.02	+ .34	+ .07	+ .14	− .34	− .10
E. G. Eskimos**	−.32	+.33	+.47	+2.08	+2.30	+1.03	+1.30	+1.85
Pecos Indians	−.06	+.34	+.14	+ .32	+ .10	+ .15	− .30	+ .08

*Weighted mean of data given for males and females was computed as bases for comparison.

**Ibid, for data of left and right, males and females.

Measurements in mm.

Furthermore, the geographical distribution of the torus is interesting. In the Eastern Aleut (living east of Atka), 61 per cent had a torus as compared to 26 per cent in the Western Aleut (those living on Atka and formerly Attu.) Thus these two populations, supposed to be geographically, linguistically, and historically differentiated,[3] exhibited a statistically significant difference in the occurrence of this feature (critical ratio of 3.4).

The effects of admixture were investigated in the Aleuts who showed the most "white" morphological characteristics, balding, beard development, light skins, light eyes, and so on. The incidence of torus formation in these near-white Aleuts was compared to that of the remaining Aleuts. In the 21 near-white Aleuts the frequency of occurrence was 43 per cent, slightly higher than for the total material, but not significantly so. The near-white group composed 19 per cent of the Western and 20 per cent of the Eastern Aleuts. Calculated in percentages, the presence of torus in the Western near-whites and Eastern near-whites was nearly identical, as it was in the total Western or Eastern

groups. Due to the small numbers, the findings in the near-white group was of no statistical significance, even assuming that the certificate "near-white" was well earned. Therefore, no conclusions can be made about the effect of admixture on torus formation in this population.

Since the torus appeared in a fairly large number of Aleuts of all ages, and genealogies were available, the data were submitted to Osborne[4] to investigate the mode of inheritance. His analysis points out that torus mandibularis depends upon genetic factors for its expression in this population and it offers the working hypothesis that its presence depends upon a single major gene, whereas the degree of penetrance is determined by two or more modifiers, cumulative in effect. This hypothesis is based on the available evidence, but a larger series is needed to confirm its accuracy.

This finding is helpful for evaluating the statement of Weidenreich[5] that Sinanthropus may have been directly related to the now living Mongoloids. If torus mandibularis is dependent upon genetic factors, then its presence in the early Chinese fossil horizon and the living Mongoloids may indicate genetical continuity.

In addition to variations in frequency of torus mandibularis in the two groups, differences in many other characteristics were found, notably in some morphological aspects of the teeth, namely the Y5 pattern[6] of the mandibular first molar, +5 pattern of the mandibular second molar, Fig. 3, tricuspal pattern of the mandibular second premolar and the quadricuspal formation of maxillary second and third molars and degree of shovel shape[7] of the maxillary incisors (Fig. 4).

The level of statistically demonstrable significance between Eastern and Western Aleuts for these differences was low, in part due to the small numbers in the series, wherefore chance effects prevented classifying the differences as valid.

Findings concerning the occlusion pointed out that in general the incidence of malocclusion, due to a disharmonious development of the maxillae, was low. However, one particular type of malocclusion, characterized by an excessive degree of mandibular prognathism causing a facial deformity, was found. This type (Class III) is rare among whites, and the type most frequently found in whites, whereby the mandible appears to be retrognathic (Class II), was absent in the Aleuts.

The incidence of excessive mandibular prognathism showed a significant difference within the Aleut population. It was found exclusively in the Eastern Aleuts, occurring in 13 per cent of this populace; when contrasted with the Western Aleuts, the critical ratio was 2.3.

Inspection of the genealogies for this trait showed a familial distribution and the genetic study of Mr. Osborne resulted in the hypothesis that a single recessive gene suffices to explain the incidence of excessive mandibular prognathism in certain Eastern Aleut families. One should bear in mind that the particular mode of inheritance found in respect to torus mandibularis and excessive mandibular prognathism, applies only to this Aleut population, and cannot be carried over to other populations without verification.[8]

On the basis of dentition alone one can only substantiate the East-West difference with regard to torus mandibularis and excessive mandibular prognathism; influence of the other factors cannot be properly evaluated. However, findings in the dentition support the anthropological, cultural, archaeological,

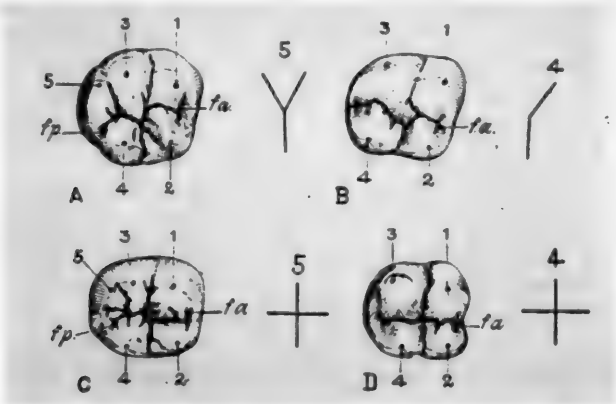


Fig. 3.—Fissure patterns of mandibular molars (from Hellman, courtesy Mrs. E. Hellman Bull).

and linguistic findings[3] and thus help to clarify the picture. Therefore, data should be presented for each group separately and not together, since we are dealing with two different populations. The examples given may have illustrated the possible value of dentition as a potential tool of differentiation within populations.

We will now consider some general aspects of the dentition as a criterion of race. Most studies to date have dealt with populations of recent or ultimate

Fig. 4.—Shovel-shaped incisors, thickening of the marginal ridges resulting in a ridge and fossa formation (keilo-kollo morphy). (Specimens courtesy Peabody Museum, Harvard University).

Mongoloid origin.[9] Results have been encouraging so far and it is generally believed that the "Mongoloid" dentition is rather outstanding with its incidence of shovel shape of incisors, lack of Carabelli's cusp of maxillary molars and presence of torus mandibularis. Data thus far published for White and Negro teeth have not shown such a distinct pattern. But even assuming that Mongoloid, white and Negro teeth can be clearly separated, we still have to consider the variations within these races. We are always dealing with subraces and composite subraces,[10] whereas the primary races are abstractions or generalizations and a pure Mongoloid does not exist. Therefore, the determination of these variations in racial subgroups by future investigators would be of great value. That will ultimately determine the value of the dentition in racial studies.

In order to give just one example of the variation within the Mongoloid group, as defined above, we will compare mesio-distal crown length of maxillary teeth in Aleuts, Japanese,[11] Chinese,[12] Javanese,[13] East Greenland Eskimos[14] and Pecos Indians[15] to those of "whites" (Swedes[16]). In order to enable this comparison, mean diameters for both sexes combined were computed if not given in the original publications, utilizing the formula for the "weighted mean." Considering all groups shown in Table I, the incisor measurements follow a general pattern, although to varying degrees. The first incisors are consistently narrower than in whites, and the second incisors consistently broader than those of whites. Therefore the four maxillary incisors are more nearly of the same absolute and relative size in these groups as contrasted to whites and this is seemingly a characteristic for Mongoloids as a group. However, no common trend for canines and premolars can be demonstrated. The three maxillary molars are smaller, with the exception of all three East Greenland Eskimo molars, the $M_1 + M_3$ of Pecos Indians and M_1 of Javanese. The greatest differences in comparison to whites are exhibited by the second and third molars, since they are smaller to a more marked degree than the first molars.

Notwithstanding the Eskimoid affinity of the Aleuts,[3] no similarity in mesio-distal tooth diameters between East Greenland Eskimos and Aleuts can be found. The East Greenland series of tooth measurements is very small and further clarification of the lack of relationship is needed. In the first place, variation within the Eskimo group should be assessed and thus established whether a difference between the Alaskan and Greenland Eskimos exists. If such a difference were present, a closer relationship between tooth size in Aleut and Alaskan Eskimo may be expected, because the Aleut is closer related to the Alaskan than to the Greenland Eskimo.

The scope of this comparison is limited because of the lack of additional statistically treated material and our conclusions at present have to remain rather vague for this reason. Despite the fact that most dental research is conducted on whites, the only statistically analyzed data concerning mesio-distal tooth diameter can be found in an orthodontic study of Seipel.[16] Our attention should therefore be directed toward more study of morphological and metric variations in the dentition of man, in order to obtain data useful in the determination of common patterns and their variations in subgroups of the same racial stocks.

CONCLUSIONS

1. The evidence gathered in the study of the Aleut suggests that torus mandibularis depends for its presence on genes rather than functional stress.

2. The multitude of features expressed in the dentition may be used to show differences within populations or between neighboring populations, even in cases where the interpopulation differences are hard to establish, provided that large enough samples are obtained to make possible a statistical evaluation of the difference.

3. A great many detailed studies of the dentition in different racial groups are needed before the dentition can be used as a reliable criterion of race.

The author expresses his gratitude to Drs. Garn and Hurme (Research Fellow in Anthropology, and Director of Clinical Research, Forsyth Dental Infirmary) for their help in the preparation of this manuscript.

Tooth measurements and statistical analysis on Aleut material by Dr. P. K. Koski, former Fellow in Orthodontics, Forsyth Dental Infirmary.

REFERENCES

1. Dahlberg, A. A.: The Changing Dentition of Man, *J. A. D. A.* **32**: 676, 1945.
2. Hrdlicka, A.: Mandibular and Maxillary Hyperostoses, *A. J. Phys. Anthr.* **28**: 1, 1940.
3. Laughlin, W. S.: The Physical Anthropology of 3 Aleut Populaces; Attu, Atka, and Nikolski. Doctorate Thesis, Harvard University, 1949.
4. Osborne, R.: Personal communication.
5. Weidenreich, F.: (From Hooton, E. A.) Up From the Ape, revised ed., New York, 1946, The Macmillian Co., Part IV, p. 304.
6. Hellman, M., Racial Characters in Human Dentition, *Proc. Am. Phil. Soc.* **67**: 157, 1928.
7. Hrdlicka, A.: Shovel-shaped Teeth, *A. J. Phys. Anthr.* **3**: 429, 1920.
8. Muller, H. J.: Progress and Prospects in Human Genetics, *A. J. Human Genetics* **1**: 1, 1949.
9. Coon, C. S., Garn, S. M., and Birdsell, J.: Races—A Study of the Problems of Race Formation in Man, Springfield, Ill., 1950, Charles C Thomas, p. 110.
10. Hooton, E. A.: Up From the Ape, revised ed., New York, 1946, The Macmillan Co., Part V, p. 568.
11. Yamada: Tooth Sizes of Japanese (quoted by Hosaka[12]).
12. Hosaka, T.: Statistische Untersuchungen über die Zähne bei Chinesen mit besonderer Berücksichtigung der Rassenunterschiede, *J. Oriental Medicine* **24**: 1065, and **25**: 348, 1936.
13. Mijsberg, W. A.: On Sexual Differences in the Teeth of the Javanese. Koninklijke Academie van Wetenschappen, Amsterdam. Proceedings meeting Oct. 31, 1931, **34**: 1111, 1931.
14. Pedersen, P. O.: The East Greenland Eskimo Dentition, Meddelelser om Grønland, **142**, 1949.
15. Nelson, C. T.: The Teeth of the Indians of Pecos Pueblo, *A. J. Phys. Anthr.* **23**: 261, 1938.
16. Seipel, C. M.: Variation of Toothposition, Lund, 1946, Hakan Ohlssons Boktryckeri, p. 50.

DOLORIMETRIC EVALUATION OF DENTAL PATIENTS

Preliminary Report Based Upon Determinations With a Radiant Heat Pain Threshold Apparatus

AUSTIN H. KUTSCHER, B.A., D.D.S., AND NEAL W. CHILTON, B.S., D.D.S., M.P.H

Division of Research, Hannah and Harry Posner Research Laboratory, School of Dental and Oral Surgery of the Faculty of Medicine, Columbia University, New York, N. Y.

THE PROBLEM of pain is of paramount importance to all branches of professional practice. This investigation, performed on 168 dental patients, was concerned with the human pain threshold and also its relation to reaction to pain, fear of pain, and the individual's estimate of his own pain threshold. Each of the subjects studied was well known to one of the investigators by reason of frequent and repeated visits to the dental office over a period of years.

No method of accurately evaluating pain sensation was available until the Hardy-Wolff-Goodell pain threshold apparatus and the technique of its application were introduced in 1940.[1, 2] In this technique which has been used extensively since that time,[3-13] pain thresholds are determined by focusing the radiant energy of a 500 watt projection bulb through an electrically timed solenoid shutter for 3 seconds onto the skin (in the present study the dorsum of the hand was used) which has been blackened with India ink to assure complete and uniform absorption of radiation. The pain threshold, measured in millicalories per second per square centimeter of exposed skin, is considered the amount of heat required to provoke a sensation of heat, finally "rising" or "swelling" to a distinct stab or prick of pain at the end of the 3 second exposure.

In their original paper, Hardy, Wolff, and Goodell[1] using themselves as trained subjects, found that their own pain thresholds ranged from 229 millicalories to 233 millicalories, which they considered uniform. Using the Hardy-Wolff-Goodell technique, Schumacher, Goodell, Hardy, and Wolff[3] studied 150 untrained subjects and found the pain threshold to vary from 173 millicalories to 252 millicalories (average 206 millicalories). They concluded that the pain threshold in man was uniform and not related to the individual's estimate of his own pain threshold. Since this uniformity was in contrast with routinely exhibited wide individual variations in the reaction to pain, they also concluded that individual reactions to pain are not the result of variations in the pain threshold. Chapman and Jones,[4] testing 200 untrained subjects, found the range of pain thresholds was from 175 millicalories to 462 millicalories (average 305 millicalories). Potelunas, Meixner, and Hardy,[8] using 65 untrained subjects in their study of skin diseases, found the pain threshold of normal skin to vary from 167 millicalories to 329 millicalories (average 235 millicalories).

This study was supported by a grant-in-aid from the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

Received for publication, June 19, 1951.

The characteristics and responses of the untrained subjects in the study herein described were well known by the investigators. This was not the case in the aforementioned large studies.

METHODS

The 168 patients included in this study, aged 10 to 60 (56 men and 112 women), were oriented in the use of the Hardy-Wolff-Goodell pain threshold apparatus (Dolorimeter) and their pain thresholds were determined by the Hardy-Wolff-Goodell technique. These subjects were considered untrained* according to the Hardy-Wolff-Goodell terminology, although no subject was included in the study who did not feel confident of his ability to identify the "end point."

Prior to determining the pain threshold, each patient was evaluated as to his: (1) reaction to pain; (2) fear of pain; and (3) estimate of his own pain threshold and susceptibility to pain. These evaluations were based upon the individual's actions before, during, and after such routine dental procedures as are usually associated with pain, i.e., injection of a local anesthetic, scaling near sensitive gingival tissues, operative dentistry, etc.

RESULTS

Study I. Range of the Pain Threshold.—The range of the pain threshold in man was studied from the individual determinations of the 168 patients. The pain thresholds of the 168 untrained subjects tested ranged from 178 millicalories to 350 millicalories with a median value of 223.5 millicalories. The quartile range was from 202 millicalories to 253 millicalories. One patient was recorded at 450 millicalories. The distribution of the pain threshold in these patients is found in Table I and in Fig. 1.

Study II. Relation of Pain Threshold to Reaction to Pain.—The pain threshold determinations of the three groups are summarized in Table II. Patients who reacted to painful stimuli under dental treatment by more than usual movement of the head or hand, etc., or by outcry, were categorized as displaying more than the average reaction to pain (Group A); patients who remained totally unperturbed and impassive to noxious stimuli were categorized as manifesting less than average reaction to pain (Group C); and patients relatively between these limits of reaction were classified as having shown average reaction to pain (Group B).

Study III. Relation of Pain Threshold to Fear of Pain.—The pain threshold determinations of the three groups are summarized in Table III. Patients were classified (according to their comments, hesitancy, cooperation, and physiological fear pattern of sweating, rapid pulse, dilation of pupils, etc.) as to their routinely manifested fear of pain as: less than average (Group A); average (Group B); or more than average (Group C).

Study IV. Relation of Pain Threshold to Subject's Own Estimate of Pain Threshold.—The pain threshold determinations of the three groups are sum-

*Trained subjects are those who have been studied repeatedly.

TABLE I

PAIN THRESHOLD OF 168 UNTRAINED DENTAL PATIENTS IN MILLICALORIES AS DETERMINED BY THE HARDY-WOLFF-GOODELL DOLORIMETER

PAIN THRESHOLD IN MILLICALORIES	NUMBER OF PATIENTS
175-184	7
185-194	12
195-204	29
205-214	17
215-224	21
225-234	12
235-244	12
245-254	21
255-264	7
265-274	9
275-284	7
285-294	6
295-304	2
305-324	2
325-350	3
450	1
Total	168

TABLE II

PAIN THRESHOLDS OF 168 UNTRAINED DENTAL PATIENTS, GROUPED ACCORDING TO THEIR REACTION TO PAIN ASSOCIATED WITH DENTAL PROCEDURES

GROUP	NO. OF PATIENTS	RANGE OF PAIN THRESHOLDS (IN MILLI-CALORIES)	MEAN PAIN THRESHOLD (IN MILLI-CALORIES)	STANDARD DEVIATION (σ)*
Less than average reaction to pain	60	450 to 179	246	49
Average reaction to pain	70	350 to 179	223	31
More than average reaction to pain	38	278 to 178	224	44

*Standard deviation $\sigma = \sqrt{\frac{\Sigma x^2}{N} - \bar{x}^2}$, where x = pain threshold of each individual; $\bar{x}$ average (mean); Σ = sum of; and N = number of subjects.

TABLE III

PAIN THRESHOLDS OF 168 UNTRAINED DENTAL PATIENTS, GROUPED ACCORDING TO THEIR FEAR OF PAIN ASSOCIATED WITH DENTAL PROCEDURES

GROUP	NO. OF PATIENTS	RANGE OF PAIN THRESHOLDS (IN MILLI-CALORIES)	MEAN PAIN THRESHOLD (IN MILLI-CALORIES)	STANDARD DEVIATION (σ)*
Less than average fear of pain	53	450 to 179	235	30
Average fear of pain	71	350 to 178	236	39
More than average fear of pain	47	290 to 181	222	29

*See footnote to Table II.

TABLE IV

PAIN THRESHOLDS OF 168 UNTRAINED DENTAL PATIENTS, GROUPED ACCORDING TO THEIR OWN ESTIMATION OF THEIR PAIN THRESHOLD

GROUP	NO. OF PATIENTS	RANGE OF PAIN THRESHOLDS (IN MILLI-CALORIES)	MEAN PAIN THRESHOLD (IN MILLI-CALORIES)	STANDARD DEVIATION (σ)*
Estimated own pain threshold as high	12	450 to 186	259	64
Estimated own pain threshold as average	110	350 to 178	230	37
Estimated own pain threshold as low	47	292 to 185	229	31

*See footnote to Table II.

marized in Table IV. Patients were asked to subjectively evaluate their own pain threshold or inherent susceptibility to pain as: higher than average (Group A); average (Group B); or lower than average (Group C).

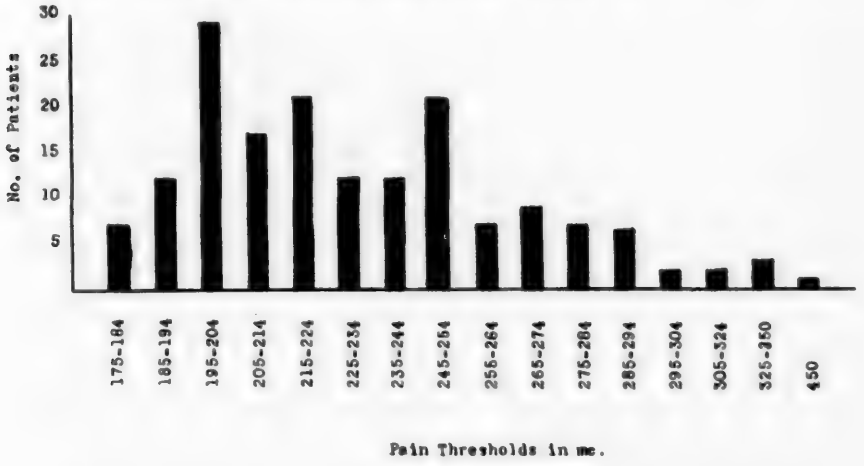


Fig. 1.—Distribution of the pain thresholds of 168 untrained dental subjects.

DISCUSSION

The mean pain threshold of 231 millicalories for the 168 untrained subjects tested in this study is not very different from figures obtained by previous investigators.[3, 4, 5] The distribution of the pain thresholds in the 168 subjects studied was positively skewed (Fig. 1). Similar asymmetrical distributions have also been noted previously.[4, 5] This may perhaps be explained by differential pain perception at lower intensities than at higher intensities. Thus, an increase of 10 millicalories from 140 to 150 millicalories may not register the same as an increase of 10 millicalories from 240 millicalories to 250 millicalories. This phenomenon has also been noted in other asymmetrically distributed characteristics of randomly sampled subjects.

Hardy, Wolff, and Goodell[9] found that the trained subject could discriminate 21 steps of pain sensation between the pain threshold and the maximum intensity of pain which could be discerned by the subject (ceiling intensity). Untrained subjects, on the other hand, are able to discern only one-half as many steps in pain intensity between the pain threshold and ceiling. Thus, there are only 10½ divisions (dols) in the Hardy-Wolff-Goodell dol scale of pain intensity[9, 10] which is reproduced in Table V.

Subjects with a pain threshold of 200 millicalories felt considerable pain when exposed to a 242 millicalories stimulus. Such a 42 millicalories increment (3 dols) is equivalent to the pain experienced during the second quarter of the first stage of labor.[5] Yet 242 millicalories was found to be the pain threshold of other individuals. Subjects with a pain threshold higher than 242 millicalories feel no sensation of pain at all when a 242 millicalories stimulus is

TABLE V

HARDY-WOLFF-GOODELL DOL SCALE OF PAIN INTENSITY[9]

TOTAL NO. MILLI-CALORIES ABOVE PAIN THRESH-OLD	INCRE-MENT IN MILLI-CALORIES	NO. OF STEPS ABOVE PAIN THRESH-OLD	NO. OF DOLS	TOTAL NO. MILLI-CALORIES ABOVE PAIN THRESH-OLD	INCRE-MENT IN MILLI-CALORIES	NO. OF STEPS ABOVE PAIN THRESH-OLD	NO. OF DOLS
7	7	1	½	80	10	11	5½
14	7	2	1	90	10	12	6
21	7	3	1½	100	10	13	6½
28	7	4	2	115	15	14	7
35	7	5	2½	130	15	15	7½
42	7	6	3	145	15	16	8
49	7	7	3½	160	15	17	8½
56	7	8	4	175	15	18	9
63	7	9	4½	205	30	19	9½
70	7	10	5	265	35	20	10
				465	200	21	10½

delivered. Also, a 250 millicalories stimulus was considerably more painful to a subject with a 200 millicalories pain threshold than to a subject with a 242 millicalories pain threshold.

When the range of pain thresholds recorded in this study (170 millicalories to 350 millicalories) and other studies[3, 4, 8] is examined in relation to the above dol scale of pain intensity, it seems that the spread of the individual pain threshold determinations indicates such a lack of uniformity of the pain threshold in man with respect to radiant heat pain stimuli as to be of definite clinical importance. A stimulus equal to the number of millicalories constituting the above range of pain thresholds, when added to the number of millicalories constituting a person's pain threshold is great enough to produce pain of severe intensity when applied to that particular individual. It is evident also that radiant heat stimuli of different intensities will produce varying sensations in different individuals, depending upon their original pain threshold.

The distribution of the individual pain thresholds of a group of trained subjects numbering as many as 168 subjects has not been reported but is at present under investigation by the author.

Examination of the data in Tables II, III, and IV discloses that there is marked overlapping of the individual pain threshold determinations between the groups in each study. There was little relationship between the pain threshold as measured by radiant heat stimuli and (1) the individual patient's reaction to pain felt during dental procedures, (2) his subjective fear of pain, or (3) his own estimation of his pain threshold. As possible explanation for these findings it is suggested that: (1) human organisms have developed differing socially acceptable individual and mass behavior patterns relative to pain; and (2) pain thresholds to radiant heat stimuli applied to the skin of the back of the hand may not exactly correspond with pain thresholds to certain other stimuli (i.e., derived from dental procedures) in any individual and from one individual to the next.

SUMMARY

1. The pain thresholds of 168 dental patients to radiant heat stimuli applied to the skin of the hand were determined by the Hardy-Wolff-Goodell technique.

2. The pain thresholds of these 168 untrained individuals were asymmetrically distributed and differed among the subjects tested.

3. It was not possible to relate the pain threshold as determined by radiant heat stimuli with reaction to pain, fear of pain, or the individual's estimate of his own pain threshold, as derived from dental procedures.

The authors express their appreciation to Dr. Albert W. Grokoest of the Department of Medicine of the College of Physicians and Surgeons, Columbia University, for suggestions during the preparation of this manuscript, and to Mrs. Joyce P. Bacher for her assistance in the performance of the experimental procedures.

REFERENCES

1. Hardy, J. D., Wolff, H. G., and Goodell, H.: Studies on Pain. A New Method for Measuring Pain Threshold: Observations on Spatial Summation of Pain, *J. Clin. Investigation* **19**: 649, 1940.
2. Wolff, H. G., Hardy, J. D., and Goodell, H.: Studies on Pain. Measurement of the Effect of Morphine, Codeine and Other Opiates on the Pain Threshold and an Analysis of Their Relation to the Pain Experience, *J. Clin. Investigation* **19**: 659, 1940.
3. Schumacher, G. A., Goodell, H., Hardy, J. D., and Wolff, H. G.: Uniformity of the Pain Threshold in Man, *Science* **92**: 110, 1940.
4. Chapman, W. P., and Jones, C. M.: Variations in Cutaneous and Visceral Pain Sensitivity in Normal Subjects, *J. Clin. Investigation* **23**: 81, 1944.
5. Hardy, J. D., and Javert, C. T.: Studies on Pain: Measurements of Pain Intensity in Childbirth, *J. Clin. Investigation* **28**: 153, 1949.
6. Kibler, R. F., and Schnaper, H. W.: The Effect of Procaine Administered Intravenously on the Cutaneous Pain Threshold, *Am. J. Med. Sci.* **220**: 199, 1950.
7. Denton, J. E., and Beecher, H. K.: Methods in the Clinical Evaluation of New Analgesics, *J. A. M. A.* **141**: 1051, 1949.
8. Potelunas, C. B., Meixner, M. D., and Hardy, J. D.: Measurement of Pain Threshold and Superficial Hyperalgesia in Diseases of the Skin, *J. Invest. Dermat.* **12**: 307, 1949.
9. Hardy, J. D., Wolff, H. G., and Goodell, H.: Studies on Pain: Discrimination of Differences in Intensity of a Pain Stimulus as a Basis of a Scale of Pain Intensity, *J. Clin. Investigation* **26**: 1152, 1947.
10. Hardy, J. D., Wolff, H. G., and Goodell, H.: Studies on Pain: An Investigation of Some Quantitative Aspects of the Dol Scale of Pain Intensity, *J. Clin. Investigation* **27**: 380, 1948.
11. Birren, J. E., Schapiro, H. B., and Miller, J. H.: The Effect of Salicylate Upon Pain Sensitivity, *J. Pharmacol. and Exper. Therap.* **100**: 67, 1950.
12. Sonnenschein, R. R., and Ivy, A. C.: Failure of Oral Antipyretic Drugs to Alter Normal Human Pain Thresholds, *J. Pharmacol. and Exper. Therap.* **97**: 308, 1949.
13. Kutscher, A. H.: The Analgesic Action of Subdamine, *New York State J. Med.* In press.

STUDIES ON THE PHYSICAL PROPERTIES OF RODENT ENAMEL

II. Density Distribution and X-Ray Diffraction Pattern

J. EDWARD GILDA*

From the Division of Dental Research and the Department of Biochemistry, the University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

SMALL rodents such as the albino rat and the Syrian hamster have often been used for the study of experimental dental caries. Gross and microscopic examinations of the anatomy of the teeth, have been made in addition to examinations of the histopathology and bacteriology of the lesion, the influence of dietary factors, and many other aspects of the subject; however, knowledge of the physical properties of molar enamel, which is destroyed by the carious process, seems to be entirely lacking.

Although no description of density distribution of animal enamel has been found in the literature, some difference between rat and human enamel might be expected. Karlström,[1] Thewlis,[2] and Deakins[3] have determined the density of small pieces of intact enamel of a number of animals including the dog, horse, ox, pig, elephant, and hippopotamus and have demonstrated that in every case animal enamel is less dense.

Only two investigations dealing with the crystallographic nature of rodent enamel have come to our attention. Jensen and Möller[4] subjected rabbit and beaver incisal enamel to x-ray powder diffraction analysis. Both showed patterns characteristic of apatite crystals although the crystallite dimensions were somewhat less than for human enamel. Sobel, Hanok, Kirshner, and Faukuchen[5] analyzed maxillary incisor enamel of rats and determined the powder diffraction patterns before and after ashing. Before heating, the enamel of both species gave apatite patterns in spite of the wide variations of composition that were encountered. On ignition, the rat enamel changed to β–$Ca_3(PO_4)_2$ while human enamel continued to show an apatite structure.

The present work is an attempt to collect and evaluate data on the density distribution and crystallographic structure of rodent molar enamel. The study was undertaken to compare it with human tissues, thereby providing additional information for evaluating the suitability of rodents in experimental caries research.

METHODS AND RESULTS

Density Distribution.—Rat molar enamel was available from a previous investigation on separation methods.[6] In brief, the teeth had been ground to pass a 200 mesh screen and had been separated into enamel and dentin by a

Presented at the 25th General Meeting of the International Association for Dental Research, Chicago, Ill., June 22, 1947 (*J. D. Res.* **26**: 457, 1947). This work was supported, in part, by a grant from the Eastman Dental Dispensary of Rochester, New York. The work is taken from the thesis which the author submitted to the Graduate School of the University of Rochester, in partial fulfillment of the requirements for the degree of Master of Science.

Received for publication June 18, 1951.

*Now at the Eastman Dental Dispensary, Rochester, N. Y.

single centrifugation according to the Manly-Hodge procedure. Several samples of the enamel were pooled to provide approximately 80 mg. of material (Sample No. I). This was composed of 88.0 per cent enamel, 11.6 per cent junction-particles, and 0.4 per cent dentin. A 45 to 60 mg. portion of the pooled enamel was separated successively at different densities over the range of 2.740 to 2.920. After the necessary observations were made, the separated portions were always added to the unused material, thoroughly mixed, and another aliquot sampled.

The Manly-Hodge technique was employed with two slight modifications. The separation apparatus was centrifuged at lower speeds, usually 1700 r.p.m., for 5 minutes. Special tared tubes were fitted with ground glass stoppers; the inner tube was flared at its upper end in order to function as a powder funnel. After centrifugation, the material suspended within the inner tube was transferred to another tared centrifuge tube and both fractions freed from the fluid by three washings with acetone. The tubes containing the powdered enamel were heated to constant weight at 100° C., were cooled in a desiccator, and were weighed. The modified apparatus eliminated the necessity of powder transfer to a watch glass for weighing and reduced the sample loss attendant. The sample weight before such fractionation and the total amount of enamel recovered (sum of both fractions) were noted in 14 separations (Table I). Enamel loss was low and frequently fell within the range of error of weighing, especially when proficiency of technique had been attained, as in Sample No. II.

Suitable mixtures of bromoform and acetone or bromoform and s-tetrabromethane were used. All separations below d = 2.85 employed stepwise addition of powder with a short centrifugation immediately following addition. After each separation, the weight of enamel sinking and enamel floating was determined. Since loss during the procedure was negligible, the fraction floating was expressed as a percentage of the sum of the two fractions (Table II). These values were plotted against density and a smooth curve (''separation curve'') drawn by inspection. The lower limit of the distribution was determined by adding the sample to a 15 c.c. centrifuge tube containing fluid of such density that all of the powder sank. The bromoform content was gradually increased until a few particles remained suspended after centrifugation. This density was taken as that at which none of the sample floated. The percentage of sample in each density interval of 0.02 over the range studied was derived from the curve.

Sample No. II was obtained from the molar teeth of 25 rats and was composed of 93.5 per cent enamel, 5.9 per cent junction particles, and 0.6 per cent dentin. Details of preparation are to be found in the previously mentioned work on methods.[6] A number of portions were separated at various densities to confirm the separation curve previously obtained. In addition, two samples of pure human enamel were fractionated at d = 2.920.

Sample No. II was divided into 6 aliquots of 45 mg. which were separated at d = 2.886 to obtain a measure of the precision of the method (Table II). The mean and average deviations were 38.8 and 1.3, respectively. The relative

TABLE I

ENAMEL RECOVERY AFTER FRACTIONATION IN TARED CENTRIFUGE TUBES

SAMPLE NO.	ORIGINAL SAMPLE WEIGHT	RECOVERY (MG.)	GAIN OR LOSS (MG.)
I	59.6	58.4	-1.2
	58.1	57.1	-1.0
	51.9	51.3	-0.6
	54.1	53.5	-0.6
	50.4	50.7	+0.3
	50.5	50.2	-0.3
	50.9	50.3	-0.6
	50.5	49.6	-0.9
II	47.1	46.8	-0.3
	43.2	43.0	-0.2
	44.4	44.4	0.0
	45.9	46.0	+0.1
	43.5	43.7	+0.2
	43.5	43.3	-0.2

average deviation of a single determination in per cent was 2.9.* The average deviation of 3 determinations made at 2.772 ± .001 was of the same absolute magnitude (1.9) although the relative average deviation was much higher.

The cumulative distribution curve (separation curve) obtained by plotting density against percentage of fraction floating is shown in Fig. 1. The density of albino rat enamel ranged from 2.720, at which point none of the material floated, to 2.920 where almost all (99.8 per cent) of the sample was lighter than the separation fluid. Data obtained with Sample No. II were in good agreement with Sample No. I except in the midportion of the curve. Values for the former fell below the curve in this region. Differences in purity of samples or in sampling procedure may have been responsible. At density = 2.922, only 44.7 per cent of the human enamel floated as compared with 99.8 per cent of rat enamel. When the data for human permanent enamel reported by Manly,

TABLE II

FRACTIONATION OF ENAMEL AT DIFFERENT DENSITIES

SAMPLE NO. I		SAMPLE NO. II	
DENSITY GM/C.C.	PERCENTAGE OF SAMPLE FLOATING	DENSITY GM./C.C.	PERCENTAGE OF SAMPLE FLOATING
2.720	0.0	2.771	4.0
2.737	1.2	2.771	3.3
2.765	3.7	2.773	5.1
2.784	7.4	2.854	15.6
2.799	10.3	2.886	37.6
2.821	12.9	2.886	37.6
2.840	20.3	2.886	37.8
2.865	32.3	2.886	38.2
2.880	42.1	2.886	39.3
2.890	57.4	2.886	42.1
2.904	81.7	2.902	73.3
2.911	86.9	*Human Enamel*	
2.920	99.8	2.922	44.7
		2.922	44.7

*Calculated according to formula $a = \frac{100. \Sigma d}{Q.N.}$

where a = relative average deviation of a single determination in per cent.
Q = arithmetical mean
N = number of determinations
d = deviation of a single determination from the mean.

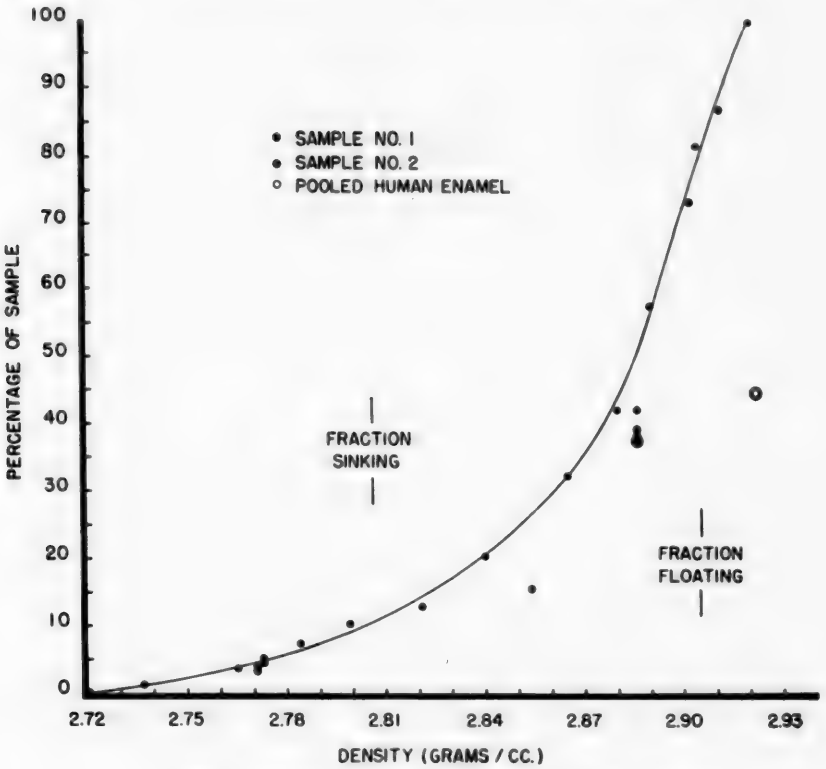


Fig. 1.—Separation curve for pooled rat molar enamel.

Hodge, and Ange[7] were plotted as a cumulative frequency distribution, our value for human enamel fell upon the extrapolated curve. This fact indicated general agreement between our respective techniques.

The percentage of sample within each density interval over the range studied is listed in Table III. The frequency distribution curve (Fig. 2) was derived by inspection from the histogram shown in the same figure and is subject to the errors inherent in such derivations. Although the curve has been extrapolated beyond the observed densities, results and subsequent discussion were confined to observations lying between 2.720 and 2.920. The curve rose slowly with increasing density to a single maximum located in the region of 2.900, and then fell off sharply to end at 2.920.

X-RAY DIFFRACTION PATTERN

Five pooled enamel samples were subjected to x-ray powder diffraction. They included 1 human, 2 rat (Nos. 9 and 10), and 2 hamster (Nos. 13 and 14) specimens.* A small amount of material was placed in a thin-walled Pyrex glass capillary tube. The tube was inserted in a Burger type camera† of 5.73 cm.

*Purity of the rodent samples is given in Tables VI and II, respectively, of a previous publication.[6]

†Manufactured by North American Phillips Company.

radius and centered with respect to the undeviated beam. Unfiltered copper radiation produced at 45 KV and 15 mA was used in every case. Length of exposure was eight hours; film employed was Eastman No-Screen. All films were processed under standard conditions.

Analysis of the patterns was divided into 2 parts: (1) measurement of position of lines and (2) estimation of relative density of lines.

The position of each line was expressed as the angle (2θ) between the undeviated and diffracted beam. Since the camera was so constructed that one mm. on its circumference was equivalent to one degree, 2θ could be determined simply by measuring the linear distance between the two points. Reproducibility of measurement was found to be ± 0.2 mm. except where lines were either very faint or comparatively broad. Under these circumstances somewhat greater variability could be expected.

TABLE III

DENSITY DISTRIBUTION OF POOLED RAT MOLAR ENAMEL (DERIVED FROM SEPARATION CURVE)

DENSITY GM./C.C.	PERCENTAGE FLOATING	PERCENTAGE OF SAMPLE WITHIN EACH DENSITY INTERVAL OF 0.02
2.720	0.0	1.4
2.740	1.4	1.9
2.760	3.3	2.6
2.780	5.9	3.5
2.800	9.4	4.8
2.820	14.2	6.3
2.840	20.5	9.1
2.860	29.6	13.9
2.880	43.5	29.4
2.900	72.9	26.9
2.920	99.8	
	Total	99.8

Relative intensity of the more pronounced lines on 3 representative patterns was determined on a recording microdensitometer. The instrument automatically recorded the photographic density of all lines in terms of milliamperes. These values were converted to time of exposure necessary to produce a corresponding density. Conversion was effected by means of a standard curve constructed in the following manner: a series of exposures to copper radiation, ranging from 4 to 40 seconds with constant increment of 4 seconds, were made on No-Screen film. The strip was run through the microdensitometer and the observed milliamperes plotted against time of exposure.

The background of general scattering was removed graphically from the densitometric tracings. Intensity of the base and peak of each line was expressed as exposure time. Since relative position of corresponding lines on the three tracings was always very similar, the intensity of the line could be expressed by the ratio, intensity of peak over intensity of base. Relative intensity of the corresponding lines in the three patterns was simplified by considering every line of the human sample as 1.00 and adjusting the other two accordingly. This method of analysis is semi-quantitative inasmuch as an internal standard was not added to the enamel sample.

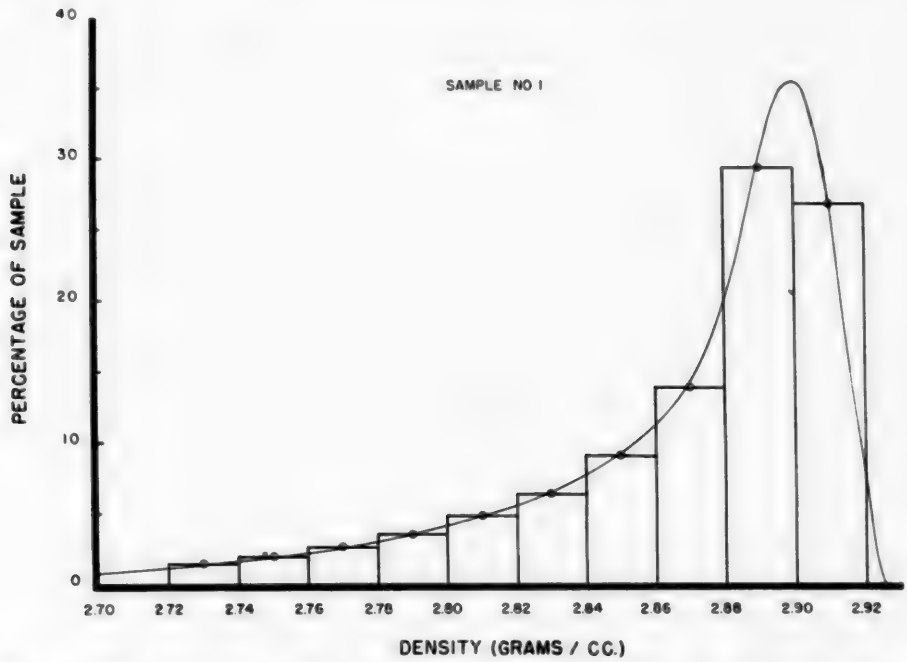


Fig. 2.—Density distribution curve of pooled rat molar enamel derived from separation curve.

The diffraction patterns of all specimens appeared to be very similar. Resolution of individual lines of human and rat patterns corresponded closely. Hamster patterns showed a slight but definite reduction in resolution over the entire strip, which is apparent in Fig. 3. The explanation for this is not known.

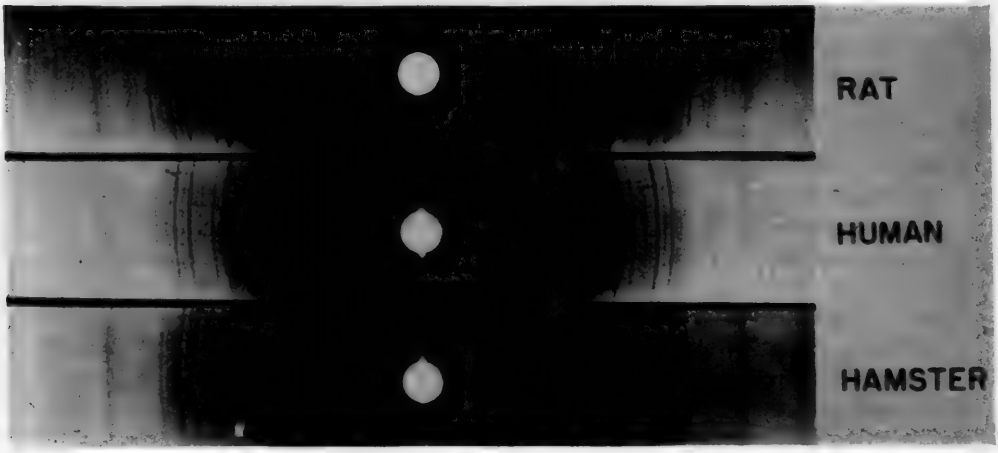


Fig. 3.—X-ray power diffraction patterns of pooled enamel.

Observations on position and intensity of the lines examined are presented in Table IV. The positions of lines in human and rat patterns in every case corresponded within the limits of experimental error (± 0.2 mm.). More variation was encountered in the hamster patterns, particularly at the larger diffraction angles. This was probably the result of less satisfactory resolution rather than of any real difference.

The 3 densitometric tracings corresponded closely in general form. Relative intensity of human and hamster patterns was almost identical. The rat pattern appeared to be somewhat more dense than the other 2, relative intensity of some lines being twice as great. Such differences are very difficult to evaluate but probably fall within the error of measurement.

In view of these findings, we believe that within close limits the portion of enamel responsible for its characteristic diffraction pattern is the same in each of the 3 species. Therefore, the inorganic salt of rodent enamel must resemble hydroxylapatite closely since Bale[8] has shown that human enamel is very similar to hydroxylapatite in structure.

DISCUSSION

Both the range of density of rat enamel and the form of its distribution curve are different from human enamel. Observations by Manly, Hodge, and Ange[7] indicate that the upper limit of human permanent enamel is quite definite at 3.00 while the lower limit is more variable but may be placed at about 2.89. Ninety per cent of the enamel is included between these limits. Two maxima are noted in the distribution curve, one at 2.97 and the other at approximately 2.90. In contrast to these data, the density of rat enamel ranges from 2.720 to 2.920, with a single maximum located at 2.90. Approximately 56 per cent of the sample is less dense than 2.890. Little can be said in explanation of a single density maximum except to point out that rat enamel resembles human deciduous enamel in this respect. Eighty-one per cent of dry deciduous enamel has a density between 2.86 and 2.98 with one maximum (2.94 to 2.96) according to Berghash and Hodge.[9]

Numerous studies have demonstrated that, in addition to water, enamel contains organic material and inorganic salts.[10] Since the dry tissue was examined, explanation of observed differences can be found either in changes of kind or proportion of the latter 2 components. Powder diffraction analysis was utilized in providing an answer to the problem. The identity of 2 samples can be established within certain well-defined limits if it can be shown that the position and density of the lines of their diffraction patterns are the same.[11] Our observation that rat and human enamel satisfy these criteria indicates the identity of the inorganic salts. Since the density of apatite is approximately 3.1, the difference in range of density distribution noted above must be the result of a greater amount of organic material in rat enamel. This conclusion finds support in the observations of Deakins and Volker[12] that rat incisor enamel contains a higher percentage of total nitrogen than does human enamel. The possibility that the organic material is dissimilar in quality remains and deserves further investigation.

TABLE IV

X-RAY POWDER DIFFRACTION DATA FOR POOLED ENAMEL SAMPLES

	ANGULAR DISPLACEMENT (2θ)					RELATIVE INTENSITY†		
IN-	HUMAN	RAT		HAMSTER		HUMAN	RAT	HAMSTER
DICES*		#9	#10	#13	#14		#9	#13
002	25.9	25.9	25.9	25.9	25.9	1.00	1.12	1.04
120	28.9	28.8	28.7	28.7	28.7	1.00	1.09	1.03
121	31.9	31.8	31.8	31.7	31.8	1.00	1.14	1.01
112	32.4	32.4	32.4	32.3	32.3			
300	33.0	33.1	33.0	33.0	32.8	1.00	1.10	.938
202	34.2	34.1	34.1	34.0	34.0			
301	35.9	35.7	35.7	35.5	35.6			
220	37.8	37.9	37.8	37.8	37.7			
130	39.9	40.0	39.9	39.6	39.9	1.00	1.11	1.02
103	40.7	40.8	40.9	40.8	40.8			
131	42.0	42.1	42.0	41.9	41.8			
113	43.4	43.2	43.2	43.0	42.9			
400	44.5	44.5	44.3	44.2	44.2	1.00	1.27	1.07
203	45.4	45.5	45.4	45.3	45.3			
222	46.8	46.7	46.6	46.7	46.6	1.00	1.37	1.07
132	48.3	48.1	48.1	48.0	47.9	1.00	1.25	1.11
123	49.5	49.6	49.5	49.4	49.4	1.00	1.78	1.12
231	50.4	50.5	50.4	50.5	50.2	1.00	1.38	1.05
140	51.4	51.4	51.3	51.1	51.2	1.00	2.36	1.04
303	52.2	52.2	52.2	52.0	51.9	1.00	1.60	1.08
004	53.2	53.4	53.3	53.3	53.2	1.00	2.11	1.19

*Indices (Miller) according to calculations of Bale.

†Intensity of each human line was considered to be unity; the corresponding rat and hamster lines were adjusted accordingly.

It might be mentioned that the method developed for density-distribution determination on small samples has some utility in the study of enamel from a single human tooth. Division of the sample into 10 or 12 aliquots would permit the necessary separations to be carried out simultaneously, thereby saving considerable time and labor. Fluorosed, carious, and hypoplastic enamels have never been examined in this manner and may have distinctly different characteristics.

SUMMARY

A method was devised for the determination of density distribution of small amounts (80 mg.) of powdered calcified tissues. It employed a modified Manly-Hodge separation tube in which successive fractionation of the same sample was performed at different densities. Albino rat enamel had a density between 2.720 and 2.920 Gm. per c.c. with a single maximum in the region of 2.90. The distribution was somewhat different from that reported for human deciduous and permanent enamel.

X-ray powder diffraction analysis demonstrated that the mineral salt in rodent molar enamel closely resembles hydroxylapatite. As a consequence of this, density differences between rat and human tissues were attributed to a relatively greater amount of organic material in rat enamel.

The author wishes to express his gratitude to Dr. Harold C. Hodge for his encouragement throughout the course of this work and for his appraisal of the completed manuscript. The author is also indebted to Mr. Herbert Mermagen, who placed at his disposal the diffraction apparatus of the Industrial X-ray Laboratory, University of Rochester and furnished valuable assistance.

REFERENCES

1. Karlström, S.: Physical, Physiological and Pathological Studies of Dental Enamel with Special References to the Question of its Vitality, Stockholm, 1931, A. B. Fahlerantz' Boktryckeri.
2. Thewlis, J.: X-ray Analysis of Teeth, *Brit. J. Radiology, N. S.* **5**: 353, 1932.
3. Deakins, M.: Changes in the Ash, Water and Organic Content of Pig Enamel During Calcification, *J. D. Res.* **21**: 429, 1942.
4. Jensen, A. T., and Möller, A.: Determination of Size and Shape of the Apatite Particles in Different Dental Enamels and in Dentin by the X-ray Powder Method, *J. D. Res.* **27**: 524, 1948.
5. Sobel, A. E., Hanok, A., Kirshner, H. A., and Fankuchen, I.: Calcification of Teeth. II. Comparison of Some Properties of Human and Rat Teeth, *J. D. Res.* **28**: 61, 1949.
6. Gilda, J. E.: Studies on the Physical Properties of Rodent Enamel. I. The Manly-Hodge Method as Applied to the Teeth of the Rat and Hamster, *J. D. Res.* **30**: 445, 1951.
7. Manly, R. S., Hodge, H. C., and Ange, L. E.: Density and Refractive Index Studies of Dental Hard Tissues. II. Density Distribution Curves, *J. D. Res.* **18**: 203, 1939.
8. Bale, W. F.: A Comparative Roentgen-Ray Diffraction Study of Several Natural Apatites and the Apatite-like Constituent of Bone and Tooth Substance, *Am. J. Roentgenology* **43**: 735, 1940.
9. Berghash, S. R., and Hodge, H. C.: Density and Refractive Index Studies of Dental Hard Tissues. III. Density Distribution of Deciduous Enamel and Dentin, *J. D. Res.* **19**: 487, 1940.
10. Armstrong, W. D.: Biochemical and Nutritional Studies in Relation to the Teeth, *Ann. Rev. Biochem.* **11**: 441, 1942.
11. Hanawalt, J. D., and Rinn, H. W.: Identification of Crystalline Materials. Classification and Use of X-ray Diffraction Patterns, *Ind. Eng. Chem. Anal Ed.* **8**: 244, 1936.
12. Deakins, M., and Volker, J. F.: Amount of Organic Matter in Enamel from Several Types of Human Teeth, *J. D. Res.* **20**: 117, 1941.

DENTINAL PROTEIN: BOUND CHOLESTEROL

R. S. LEOPOLD, PH.D., W. C. HESS, PH.D., AND W. J. CARTER, D.D.S.

Departments of Biochemistry, U. S. Naval Dental School, Bethesda, Md., and the Medical and Dental School, Georgetown University, Washington, D. C.

SEVERAL authors have mentioned that lipids are constituents of human teeth. Akamatsu[1] and Kokubun[2] used Sudan III and other Sudan dyes to demonstrate this fact histochemically in ground slices of enamel. Kokubun also mentioned that fat occurred in the dentinal portion of the tooth sections with which he worked. Using a quite different approach to show the presence of lipids, Krasnow[3] determined that enamel and dentin both had measurable lipid phosphorus content. Pincus[4] isolated, from the organic material in enamel, about 1 per cent of a fatty substance the nature of which he did not elucidate. Pincus stated that sterols were a possible constituent which had been overlooked in teeth.

The present communication deals with the presence of cholesterol as a normal constituent of the root dentin of human teeth and its extraction from the dentin.

SAMPLE PREPARATION

The samples of dentin used in this experiment were prepared in the following manner: (1) Roots of sound third molars, preserved by freezing, were severed 1 mm. below the cementoenamel junction. (2) The cementum was removed from the outer surface of each root by grinding with a diamond disc and then with a cross-cut bur. (3) The root canal walls were removed with tapered fissure burs, so that no trace of original pulp chamber remained. (4) Roots, from a minimum of 10 teeth, were broken to 16 mesh size or smaller in a steel, plunger-type mortar and pestle. A few samples of dentin of this particle size were treated with HCl; the dentin for all other samples was further reduced in size in a Mullite mortar and pestle so that all particles passed a 200 mesh standard sieve. (5) After preparation, samples of 2 to 9 Gm. of dentin powder were weighed and used without further drying, since the objective was not rigorous quantitative determination but rather quantitative recognition.

EXPERIMENTAL

Experiment I.—A sample of dentin was extracted with 30-60° B. P. petroleum ether in a Soxhlet extractor for 96 hours. When concentrated and tested the petroleum ether was found to contain no cholesterol or cholesteryl esters.

Since cholesterol and its esters were not extracted by this process, it was felt that an alcoholysis or hydrolysis (acidic or basic) might serve to free any cholesterol which was bound to the dentinal protein.

Experiment II.—A sample of dentin was extracted with 95 per cent ethanol in a Soxhlet extractor for 24 hours, the ethanol removed, and 24 hours' extraction made with ethyl ether. The solvents were mixed, concentrated under reduced pressure, and found to contain 14 mg. of total cholesterol per 100 Gm.

Received for publication, June 18, 1951, Revised by author, Aug. 6, 1951.

of original dentin, of which approximately 50 per cent was in the form of cholesteryl esters. All cholesterol and cholesteryl esters were determined by the method of Hess.[5]

Experiment III.—A 2 Gm. sample of 16 mesh dentin was demineralized with 40 ml. of 5 per cent HCl for 48 hours. The HCl solution and dentinal residue were placed in a separatory funnel and extracted with successive portions of petroleum ether. The petroleum ether contained only traces of cholesterol.

The acid residue (inner and outer fluids) of a complete demineralization of a sample of dentin, carried out at pH 1, was extracted with petroleum ether and no cholesterol was found. This was repeated with the same negative result, using combined inner and outer fluid from a second complete demineralization made at pH 1. This process of demineralization[6] was known to give nearly minimum protein destruction. From this it appeared that cholesterol and its esters would be recovered only after hydrolysis had progressed to a marked degree. The volume of acid solution used in the first part of Experiment III was also judged not sufficient for the desired result and for that reason larger acid volumes were used in subsequent experiments.

A 2 Gm. sample of 16 mesh size dentin was treated for 72 hours with 150 ml. of 2 per cent HCl at room temperature. The whole, solid and liquid, was then extracted with successive portions of petroleum ether and yielded 13.5 mg. per cent total cholesterol.

Using 200 ml. of 1 per cent HCl and a 2 Gm. sample of 16 mesh dentin for 72 hours, 7.2 mg. per cent of total cholesterol was recovered.

Results obtained with similar experiments on 2 Gm. samples of 200 mesh dentin are tabulated in Table I.

TABLE I

TIME HOURS	PER CENT ACID	VOL. ACID ML.	TOTAL CHOLESTEROL IN MG. PER CENT
72	1	200	9.6
72	2	150	21.0
72	2	150	22.5
72	5	100	20.8

Experiment IV.—A sample of dentin was boiled under reflux with 2 per cent Na_2CO_3 for 7 hours. The dentin and sodium carbonate solution were extracted with successive portions of petroleum ether. The extract was found to contain 6.4 mg. per cent total cholesterol.

Experiment V.—A sample of dentin was boiled under reflux in 2 per cent KOH for 7 hours. The dentin and KOH solution were then extracted with petroleum ether and 3.1 mg. per cent total cholesterol recovered.

Experiment VI.—A sample of dentin was placed in an aqueous solution containing 10 per cent KCl and 2 per cent K_2CO_3 and held at 65° C. for 24 hours. The dentin and solution were repeatedly extracted with petroleum ether and the extract was found to contain 13.0 mg. per cent total cholesterol.

Experiment VII.—A sample of dentin was shaken for 48 hours at room temperature in an aqueous solution containing 30 per cent KCl and 1 per cent K_2CO_3, 20 ml. of solution was used for each gram of sample. The extract was then decanted and the process repeated, using a fresh KCl,K_2CO_3 solution for an additional 48 hours. The second extract was then decanted and the liquid extracts combined. At this point the extracts were cloudy.

The combined extracts were then treated with 0.4 Gm. potassium acetate, 0.4 Gm. silica gel, and 0.4 ml. glacial acetic acid per gram of original sample weight of dentin. The solution was then shaken for 30 minutes and filtered with suction through a fine sintered glass filter. The clear solution obtained was then successively extracted with petroleum ether. The petroleum ether extract contained 24.2 mg. total cholesterol per 100 Gm. of original dentin. The silica gel and the sample residue were repeatedly extracted with petroleum ether, but no cholesterol was found in this extract.

CONCLUSIONS

1. Root dentin from normal, human third molars was found to contain approximately 24 mg. of cholesterol and its esters per 100 Gm. of dentin. The cholesterol and its esters appear to be bound to the dentinal protein.

2. Cholesterol and its esters may be recovered from human root dentin by means of either basic or acidic hydrolysis in aqueous solution followed by petroleum ether extraction, or alcoholysis with ethanol followed by ethyl ether extraction. Hydrolysis appears to be more successful than alcoholysis.

3. Weak base produces less destruction of cholesterol than strong base, and room temperature produces better yields than high temperatures.

4. Basic hydrolysis for the recovery of cholesterol is best carried out with an alkali carbonate in a solution saturated with salt, the extraction being made after neutralization of the weak base with weak acid.

5. Acid demineralization will not of itself produce sufficient hydrolysis for the liberation of cholesterol. It is necessary that sufficient acid strength be maintained so that considerable hydrolysis and solution of the protein take place.

This investigation is part of a joint project of the U. S. Naval Dental School and the Georgetown University Dental School. Part of the funds for the project were made available under Research Project NM-008-007 of the Medical Department of the U. S. Navy. The opinions and assertions herein contained are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

REFERENCES

1. Akamatsu, K.: The Occurrence of Fat in the Enamel of the Teeth, *Vierteljahrshr. für Zahnheilk.* **45**: 33, 1929.
2. Kokubun, S.: Beitrage zur Kenntnis des Auftretens von Fett in Zahnschmelz, *Transactions Japanese Path. Soc.* **21**: 687, 1931.
3. Krasnow, F.: Cholesterol and Lecithin in Teeth and Saliva, *J. D. Res.* **14**: 226, 1934.
4. Pincus, P.: Enamel Protein, *Proc. Royal Society Med.* **32**: 513, 1938.
5. Hess, W. C.: Chromatographic Separation of Cholesterol and Cholesterol Esters in Blood, *J. Lab. & Clin. Med.* **32**: 1163, 1947.
6. Losee, F. L., Leopold, R. S., and Hess, W. C.: Dentinal Protein: Recovery in Pure Form, *J. D. Res.* (In press.)

DENTINAL PROTEIN: SOME EFFECTS OF PRESERVATIVES*

W. J. CARTER,** B.S., D.D.S., R. S. LEOPOLD, PH.D., BETHESDA, MD., AND
W. C. HESS, PH.D., WASHINGTON, D. C.

Departments of Biochemistry, Naval Dental School, NNMC, Bethesda, Md., and Georgetown University, Washington, D. C.

SOME investigators report the manner of preservation of the teeth they have subjected to chemical analysis, others either use no preservative or fail to include this detail in their report. Armstrong and Brekhus[1] placed extracted teeth in 95 per cent alcohol, Deakins and Volker[2] air dried their samples of teeth, Logan[3] used fresh teeth, while Crowell, Hodge, and Line[4] immersed teeth in tap water until preliminary procedures could be applied.

No investigation of the effects of preservatives on dentinal tissues has been reported, therefore we will consider the effects of preservatives on proteins in general. The changes that occur in protein because of chemical or physical treatment have been loosely referred to as "denaturation," thus this term has many meanings and is not clearly defined. Neurath[5] stated that increase in reactivity of certain polar groups, loss of ordered structure, or tendency to aggregate are only general manifestations of denaturation. These tendencies are principally noted as a decrease in solubility and an increase in particle size. Steinhardt[6] gave the opinion that types of denaturation differ and are specific for each group of proteins and for each reagent.

It has been established that alcohol solutions of suitable percentages will precipitate and dehydrate certain proteins,[7] that chloroform because of similar behavior is also a protein denaturing agent,[7] and that formaldehyde produces a tanning effect (different type of denaturation) on collagen.[6] This investigation was made to determine what effect is exhibited on dentinal protein by reagents known to denature other proteins.

In this study samples of teeth were preserved either in alcohol, or in chloroform or in 10 per cent formalin. After a specific time in the preservative the teeth were removed and coronal dentin samples prepared. The dentin samples were analyzed, demineralized, and the resulting insoluble material analyzed and studied. The results of analyses of these samples before and after demineralization were compared with those reported for teeth preserved by quick freezing.[8]

The technique of sampling teeth, isolation of coronal dentin, drying dentin, determination of particle size of ground dentin, demineralization time and temperature, nitrogen analysis, and ash determination was that cited by Losee,

Received for publication May 14, 1951.

*The opinions or assertions in this paper are those of the authors and do not necessarily reflect the opinions or policy of the Navy Department.

**The data reported are taken from the thesis presented by Dr. Wm. J. Carter in partial fulfillment for the degree of Master of Science in Biochemistry at Georgetown University.

Leopold, and Hess,[8] with the exception that upper and lower third molars were mixed in this experiment, not kept separate. It should be noted that wherever teeth with roots open at the apices were used in any of these experiments this fact has been designated in Table I.

TABLE I

PRESERVATIVE	I. FREEZING		II. FORMALDEHYDE		III. CHLOROFORM		IV. ALCOHOL, 70%	
1. No. of days in each preservative	30	45	30*	270	9*	270	21	270
2. % Nitrogen in original dentin	3.27	3.20	3.25	3.28	3.25	3.31	3.35	3.20
3. % Ash loss of original dentin	29.40	27.90	28.50	30.20	27.84	28.43	29.34	29.78
4. Temperature of demineralizing HCl	21 C	23 C	23 C	19 C	24 C	20 C	23 C	19 C
5. % Original dentin recovered as insol. protein	15.65	15.47	18.66	18.26	13.72	14.94	15.71	12.30
6. % Ash residue of insol. material	0.60	0.60	0.59	0.00	0.70	0.37	0.00	0.00
7. % Nitrogen of insoluble material	16.24	16.10	15.62	16.50	15.87	16.22	16.30	16.30
8. % Nitrogen of original sample weight in:								
a) Insol. material	2.54	2.45	2.76	3.01	2.11	2.42	2.56	2.00
b) Inner fluid	.47	.49	.13	.06	.27	.07	.05	.52
c) Outer fluid	.17	.17	.36	.08	.59	.65	.63	.56
d) Rinses (ether free)	.06	.07	.02	.06	.25	.10	.08	.10
9. Sum of lines a, b, c, and d	3.24	3.18	3.27	3.21	3.22	3.24	3.32	3.18
10. % Original nitrogen accounted for (line 9 divided by line 2)	99.08%	96.3%	100.6%	97.9%	99.0%	97.8%	99.10%	99.3%

*One or more teeth in sample have roots open.

RESULTS

Frozen teeth, the material chosen for comparative reasons, yielded the data in the first column of Table I. In addition, the protein has certain characteristics which should be noted. It is white in color and forms a soft, fibrous, yet very friable mat upon drying. When dry it is quite light and readily acquires a static charge. Lastly, there is difficulty encountered in weighing samples of the material due to its hygroscopicity; open to the atmosphere it gains weight very rapidly. A photomicrograph of this protein, Fig. 1, appears as a rather regular particle with a fair degree of uniformity in size.

The object of this set of experiments has been to show that when teeth are preserved in various ways, the protein recovered is either the same or different. Therefore, the above is to be kept in mind for comparison with each of the following cases.

Ten Per Cent Neutral Formaldehyde Solution.—The data shown in the second column of Table I were obtained using dentin from teeth previously pre-

Fig. 1.

Fig. 2.

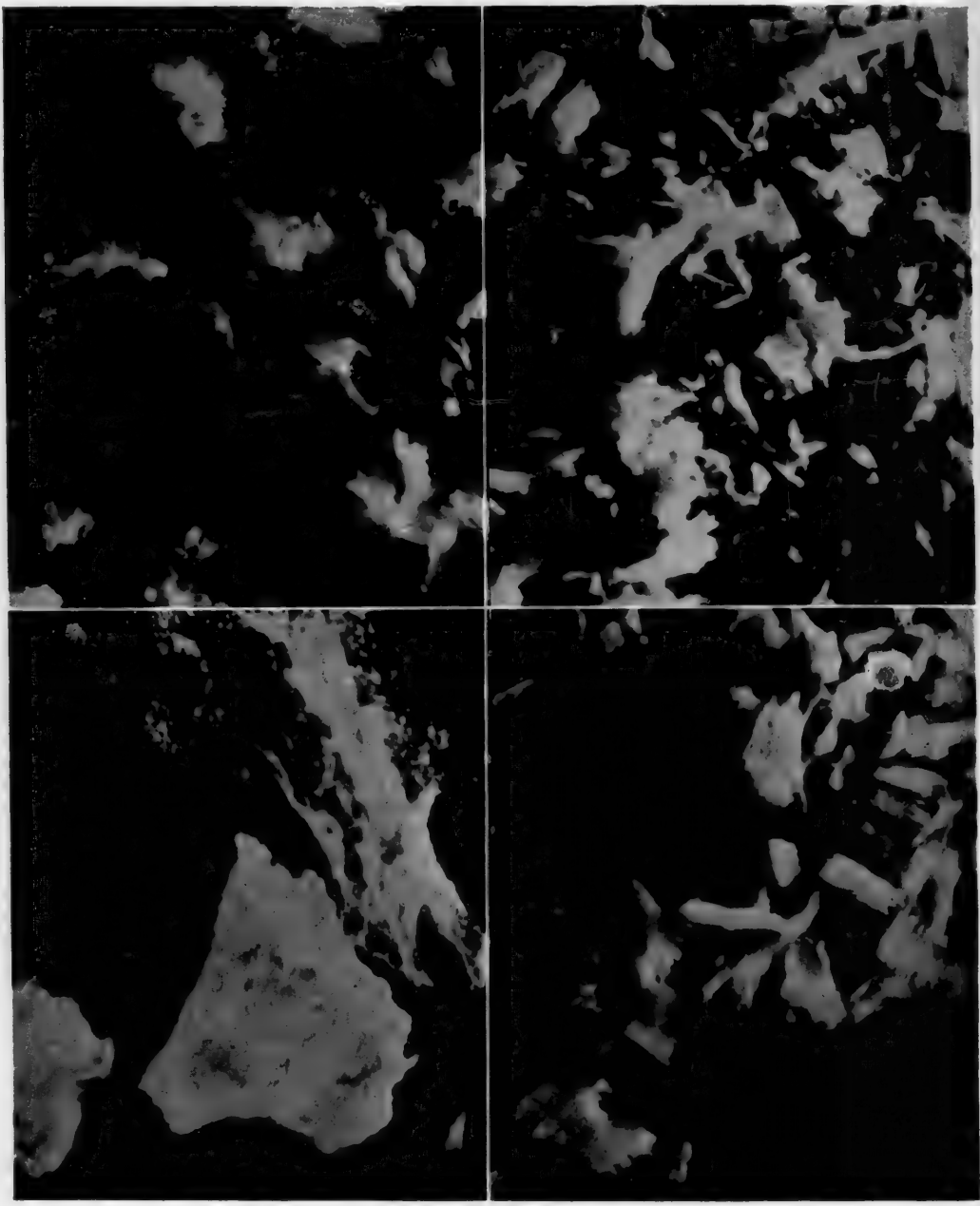


Fig. 3.

Fig. 4.

Fig. 1.—Dentinal protein from teeth preserved by freezing.
Fig. 2.—Dentinal protein from teeth preserved by formaldehyde.
Fig. 3.—Dentinal protein from teeth preserved by wet chloroform.
Fig. 4.—Dentinal protein from teeth preserved by 70 per cent alcohol.

served in formaldehyde solution. It is notable that recoveries of protein are higher by approximately 3 per cent, and that considerably less nitrogen finds its way into solution.

The recovered protein is quite dense, having a coarse, grainy appearance and feel. It is hygroscopic but not easily affected by static charges. It is apparent from the results tabulated that an increase in length of time of preservation decreases the solubility of the recovered protein. The photomicrograph of this protein, Fig. 2, shows less uniformity in particle arrangement and size than the other pictured proteins. These particles have no discernible pattern.

Wet Chloroform.—The data shown in the third column of Table I were obtained, using dentin from teeth previously preserved in wet chloroform. Here it will be noted that recoveries are from 1 to 1.5 per cent lower than the other types of preservation.

When dry, the recovered protein was a yellowish brown in color, and had the appearance of dried glue. In attempts to remove the protein from centrifuge tubes for transfer to the weighing pigs, it was found that the material adhered tenaciously to the sides of the glass. In order to obtain samples for analysis, the material had to be broken into smaller chips. This protein did not evidence the hygroscopicity nor did it obtain the static charge to the degree observed in other types of preserved samples. It may be assumed that low protein recoveries are directly due to the physical alteration of dentin in this preservative. There is some indication that here again the time of preservation decreases the solubility of the recovered protein. The photomicrograph of this protein, Fig. 3, appears as a solid, continuous phase.

Seventy Per Cent Ethyl Alcohol Solution.—The data shown in the fourth column of Table I were obtained, using dentin from teeth previously preserved in aqueous 70 per cent alcohol. The amount of recoverable protein is nearly the same as from teeth preserved by freezing. There is more nitrogenous material in solution than from other samples and the length of time of preservation tends to increase the solubility of the recovered protein.

The recovered protein was white and granular, and appeared visually as small individual beads of material. The protein was easily freed from its container, pouring like face powder. Residual amounts of the material became statically charged, and the entire portion was etxremely hygroscopic. Photomicrographically, this protein, Fig. 4, appeared as long bands with serrated edges, and possessing finer fragments dispersed throughout.

SUMMARY AND CONCLUSION

Sound, normal, upper and lower third molar human teeth were preserved in solutions of alcohol, chloroform, and formaldehyde. A comparison of methods of preservation of teeth using quick freezing as a standard has been made. The factors considered were protein recovery, nitrogen analysis, and gross microscopic appearance of recovered protein.

It is noted that denaturation does occur as evidenced in the low amounts of insoluble material in solution found in samples that were exposed to formaldehyde; also in the gross physical appearance of the protein found in samples

that were preserved in chloroform. The chemical changes brought out by nitrogen analysis are not significant, and appear only to add further weight to the assumption that dentinal protein in a heterogenous group of teeth will contain a fairly constant amount of nitrogenous materials although it may be chemically altered. All of the preservatives appeared to yield samples possessing to some degree the properties of hygroscopicity and easy acquisition of static charge.

The results brought forth in this experiment may affect the chemical and histochemical analyses reported by previous workers, since heretofore the manner of preservation has not been considered a variable.

REFERENCES

1. Armstrong, W. D., and Brekhus, P. J.: Chemical Constitution of Enamel and Dentin, *J. Biol. Chem.* **120**: 677, 1937.
2. Deakins, M., and Volker, J. F.: Amount of Organic Matter in Several Types of Human Teeth, *J. D. Res.* **20**: 117, 1941.
3. Logan, M. L.: Composition of Cartilage, Bone, Dentin, and Enamel, *J. Biol. Chem.* **110**: 375, 1935.
4. Crowell, C. D., Hodge, H. C., and Line, W. R.: Chemical Analysis of Tooth Samples Composed of Enamel, Dentine, and Cementum, *J. D. Res.* **14**: 251, 1934.
5. Neurath, H., Greenstein, J. P., Putnam, F. W., and Erickson, J. O.: The Chemistry of Protein Denaturation, *Chem. Rev.* **34**: 157, 1944.
6. Steinhardt, J.: The Chemistry of the Amino Acids and Proteins, *Ann. Rev. Biochem.* **14**: 145, 1945.
7. Hawk, P. B., Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, ed. 12, Philadelphia, 1937, P. Blakiston's Son Co., pp. 157 and 163.
8. Losee, F. L., Leopold, E. S., and Hess, W. C.: Dentinal Protein, Recovery in Pure Form. To be published.

PHYSICAL PROPERTIES OF AMALGAM AS INFLUENCED BY VARIATION IN SURFACE AREA OF THE ALLOY PARTICLES

WALTER S. CROWELL AND RALPH W. PHILLIPS

S. S. White Company, Philadelphia, Pennsylvania and Indiana University School of Dentistry, Indianapolis, Ind.

THERE has been some work done in the past by Jarabak[1] and Gray[2, 3] measuring the dimensional change on samples of amalgam alloy where the various particle sizes were separated by sieving. These studies showed a general decrease in expansion as the particle became smaller, but the differences obtained were often quite small. It is quite possible that greater variation was not shown because the fractions which are produced by sieving are not greatly different in terms of the actual specific surface. Alloy particles are usually in the form of flakes, and one dimension, the thickness, is very much smaller than the other two. Sieving separates large flakes from the small, but the difference in specific surface so produced is not great. Much more significant differences in the actual surface area can be produced by changing the thickness of the flakes.

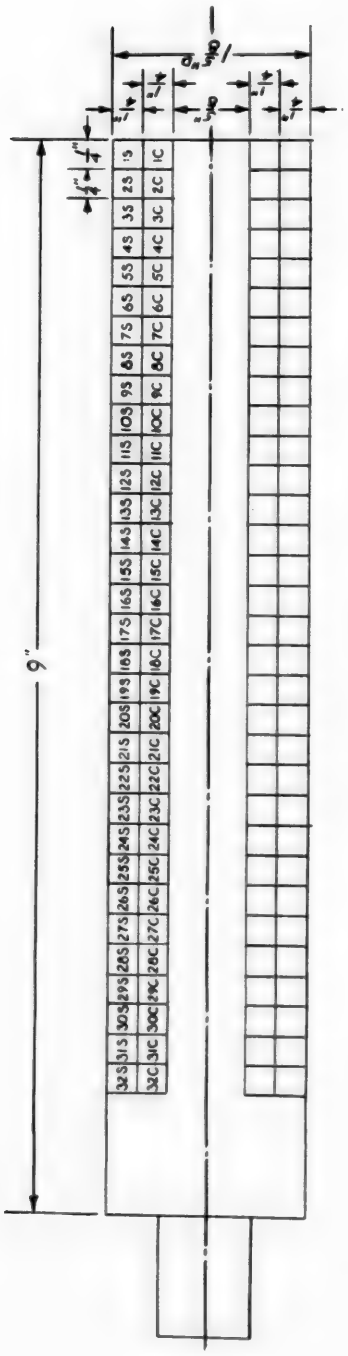
It should be pointed out that the appearance of the alloy in the bottle is not a certain indication of the "fineness" of the particles. The presence or absence of large flakes in the material makes a great deal of difference in the appearance of the alloy but may be entirely misleading as an indication of actual fineness. A relatively coarse alloy, after ball milling for a few minutes, will appear much "finer" than a fine alloy which has not been ball milled.

Obviously, an alloy consisting entirely of fine particles will react more rapidly with the added mercury and will produce a "smoother" amalgam than one consisting of coarse particles. Here again, however, clinical observation may be misleading. The readiness with which the mercury attacks the alloy particles is influenced by factors other than particle size and actual "smoothness," i.e., the presence or absence of hard particles, is not easy to observe. Grittiness or lack of "smoothness" can, of course, be observed in the laboratory when the partially set alloy is carved.

It was felt that further study should be given to the effect of specific surface, in other words, the actual surface area per unit weight of particles, upon not only the dimensional change but also the setting time, compressive strength and flow. In this study, a technique was employed in the manufacture of the experimental samples so that the specific surface could be varied by means of varying the thickness of the individual flakes. Specific surface was estimated by calculating the surface area of a sheet of alloy of the thickness determined by the feed of the cutting tool and weighing 1 oz. Troy. Physical properties were then studied on these samples which possessed widely different specific surfaces.

Presented before the Dental Materials Group at the 29th Annual Meeting of the International Association for Dental Research, March 17, 1951, French Lick, Ind. (J. D. Res. 30: 499, 1951).

Received for publication, April 19, 1951.



SKETCH SHOWING HOW INGOTS WERE CUT

Fig. 1.

PROCEDURE AND RESULTS

When specimens are prepared by hand, variations in the amount of working of the amalgam occur which often have sufficient effect on the results that the influence of factors intentionally changed is obscured. Since a slight under or over trituration will markedly alter the dimensional change and strength, it was important that each mix be triturated or nearly alike as possible. In order to eliminate some of the variables involved in hand trituration, all mixing was done by means of a mechanical amalgamator. Once the mixing time was properly established for each sample, that particular timing was used on all subsequent mixes. A 5:8 alloy-mercury ratio was employed.

It is also an established fact that properties of amalgam are influenced by variations in condensation pressure and the packing procedure. Since it is most difficult, if not impossible, to control accurately the packing thrust and the amount of work done on the alloy either by hand or by mechanical condensation, all specimens in this study were prepared by means of an automatic packing device. No mercury was expressed after trituration, but the entire mix was immediately placed in the mold and a static load of 4,000 pounds per square inch (2.8 kg. per millimeter^2) was applied for two minutes. The specimen, 10 mm. by 5 mm. for the dimensional change and compressive strength tests and 8 mm. by 4 mm. for the flow test, was then trimmed smooth with a razor blade and removed from the mold. The tests were carried out in accordance with A.D.A. specification No. 1. A Tinius Olsen machine was used for determining the crushing strength, the load being applied at the rate of 650 pounds per minute. At least five specimens were run for each sample, and any specimen which varied by more than 15 per cent from the average was omitted from the data and additional tests made. Since other investigations[4, 5] have emphasized the importance of a test for early compressive strength, one-hour specimens were tested as well as the more common 24-hour test.

With a precise method of preparing specimens it was soon found that variations in properties did occur when different ingots of the same chemical composition were tested and even in cuttings of a given thickness made from different parts of the same ingot. There were factors which had to be taken into consideration before experimental samples could be prepared in which the surface area of the particle was the only known variable.

Such results are not entirely unexpected to those who are familiar with the published data on the metallurgy of amalgam alloys. The system silver, copper, tin, and zinc is a very complicated one and the melting range of alloys which are commercially useful is wide. Murphy's data on the alloys of silver and tin show that the eutectic arrest, the temperature at which the eutectic of Ag_3Sn and tin solidifies, can be detected in alloys which are well into the solid solution range of composition and are analogous to commercial alloys.[6] Microscopic examinations of cross sections of commercial ingots, cast under conditions where cooling and solidification are as rapid as the production of sound ingots will permit, show the dove gray eutectic of Ag_3Sn + Sn in larger and larger quantities as the center of the ingot is approached.

In order to study the variations in properties of cuttings from different parts of a single ingot of known chemical composition, a 100-ounce ingot of alloy approximately 1⅝ inches in diameter by 9 inches long was prepared.* This ingot was mounted in a lathe and reduced to cuttings by taking 2 cuts about ¼ inch deep. The first cut across the ingot will be referred to as the skin and the second as the center. The cuttings from each ¼ inch of the axial length of the ingot were collected separately. The feed of the tool was changed after each ¼ inch cut until chips of four different thicknesses were produced. The thicknesses chosen were approximately .0005 inch, .0007 inch, .0010 inch, and .0014 inch. The sequence was then repeated so that every fourth sample was made up of chips of the same thickness. The sketch in Fig. 1 illustrates the procedure employed. For example, in cutting this ingot, the skin cuts of numbers 8S, 12S, 16S, and 20S, etc., were all of one particle size while cuts numbered 9S, 13S, 17S, and 21S, etc., had the same thickness. The same procedure was followed for preparing the center cuts and those individual cuts are represented in the sketch as "C" numbers. The samples were annealed for 7 days at 60° C. and tested individually for expansion, setting time, and flow.

TABLE I

INGOT NO. 1. VARIATION OF EXPANSION WITH LOCATION OF CUT SPECIFIC SURFACE—573 SQUARE INCH PER OUNCE TROY

DISTANCE FROM BOTTOM INCHES	24 HOUR EXPANSION MICRONS PER CM.	
	SKIN	CENTER
½	8.2	0.8
1	7.0	3.2
1½	-0.4	0.4
2	-1.5	3.0
3	1.2	--
4	--	3.2
5	1.0	--
6	--	3.0
7	1.8	--
8	--	3.8
9	1.5	--

Dimensional change and setting time were found to give the most delicate indications of similarity or differences between the samples. In Table I can be seen the data obtained on one thickness of chip when cut from different parts of the same ingot. It is evident that the bottom end of the ingot differs from the remainder and that the outer layer, or skin, differs from the center. Similar tests were made with chips of three other thicknesses. From these data it was possible to determine the portion of the ingot where the properties of a given particle size appeared to be most uniform. For example it is apparent (Table I) that the properties of the cut having a specific surface of 573 square inches per ounce appear to be much more uniform when cut from the middle or top of the ingot rather than from the bottom. Having established the general location in the ingot where properties of each size particle seemed to be most consistent,

*The formula of the ingot was: silver—70 per cent, copper—2.5 per cent, tin—25.5 per cent, and zinc—2 per cent.

samples of adjoining cuts taken from this portion of the ingot were tested for all properties except compressive strength and results then compared on the assumption that chip thickness was the only variable.

The data from these tests are shown in Table II. It should be kept in mind that in Column 1 the smaller the particle thickness, the greater is the surface area per ounce of alloy. The setting time was measured by means of a penetrometer. These data indicate that as the specific surface increases, the expansion, setting time, and flow all decrease on samples taken from the skin of the ingot. It can be seen that the data were generally quite similar when adjoining cuts taken from the center of the ingot were tested. The data on dimensional change resemble those reported by Jarabak on his alloys A and B.[1] He shows that the fractions of these samples of alloy, obtained by sieving, differ in expansion with the expansion falling off as the size of the particles of these two alloys is reduced. The effect of changing the particle size of his alloy C by sieving was much less, probably because the sieved fractions of this particular sample did not differ greatly in their specific surface.

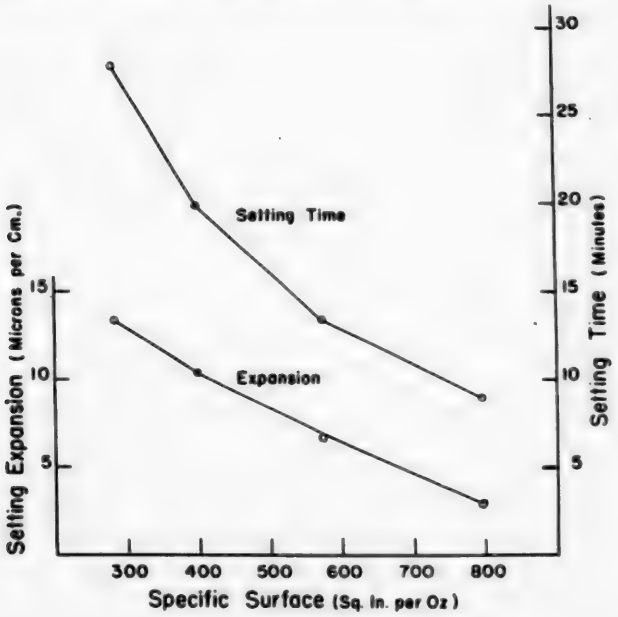


Fig. 2.

In order to check these data and to make tests on samples involving a greater portion of the ingot, another ingot was prepared at a later date. The same cutting procedure was employed as before. After testing individual samples, those cuts of a given chip thickness which had closely similar properties were mechanically mixed and the mixed samples then retested for the previous properties as well as the one-hour and twenty-four-hour compressive strength (Table III).

TABLE II

INGOT NO. 1. EFFECT OF SPECIFIC SURFACE ON PROPERTIES TESTS MADE ON ADJOINING CUTS

SPECIFIC SURFACE SQUARE INCH PER OUNCE TROY	SKIN			CENTER		
	EXPANSION (MICRONS PER CM.)	SETTING TIME (MINUTES)	FLOW PER CENT	EXPANSION (MICRONS PER CM.)	SETTING TIME (MINUTES)	FLOW PER CENT
287	13.8	51	6.1	12.7	52	5.3
401	4.7	29	4.2	10.5	30	4.1
573	1.2	20	3.1	4.2	19	3.5
801	1.1	14	2.6	1.4	13	4.1

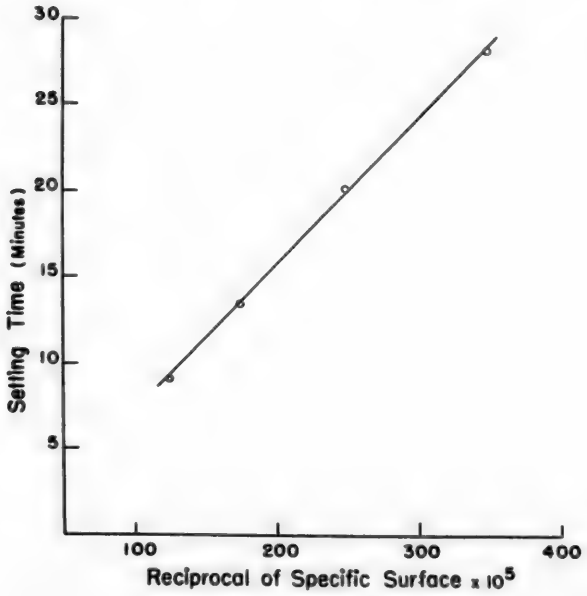


Fig. 3.

When setting expansion and setting time are plotted against the estimated surface area (Fig. 2) it will be seen that both of these properties vary regularly with the increase of specific surface. Setting time and setting expansion become smaller as specific surface is increased. Expansion appears to be a linear

TABLE III

PROPERTIES OF INGOT NO. 2. TESTS MADE ON CUTS POOLED FROM THROUGHOUT INGOT

SPECIFIC SURFACE SQUARE INCH PER OUNCE TROY	SETTING EXPERIMENT 24 HOUR (MICRONS PER CM.)	SETTING TIME (MINUTES)	COMPRESSIVE STRENGTH (P.S.I.)		FLOW PER CENT
			1 HOUR	24 HOUR	
801	2.9	9	11,600	47,000	3.8
573	7.0	13½	8,900	43,500	3.7
401	10.4	20	7,200	41,000	3.6
287	13.6	28	5,800	41,000	5.4

function of specific surface; setting time is not. However, when setting time is plotted against the reciprocal of the specific surface (Fig. 3) the curve approaches a straight line.

A few calculations on the equation for heterogeneous reaction velocity should indicate why such a graph might be anticipated. The reaction between mercury and alloy chips is a heterogeneous one and these results should approximately correlate with the Noyes-Nernst equation for such reactions.

1. $\frac{dx}{dt} = KS\ (a\text{-}x)$
2. $KST = \log \frac{a}{a\text{-}x}$
3. $T = \frac{1}{KS} \log \frac{a}{a\text{-}x}$
4. $\log\ (a\text{-}x) = \log a - KTS$

a = initial quantity of mercury

x = mercury consumed in time T

S = Surface area of solid phase

K = proportionality constant

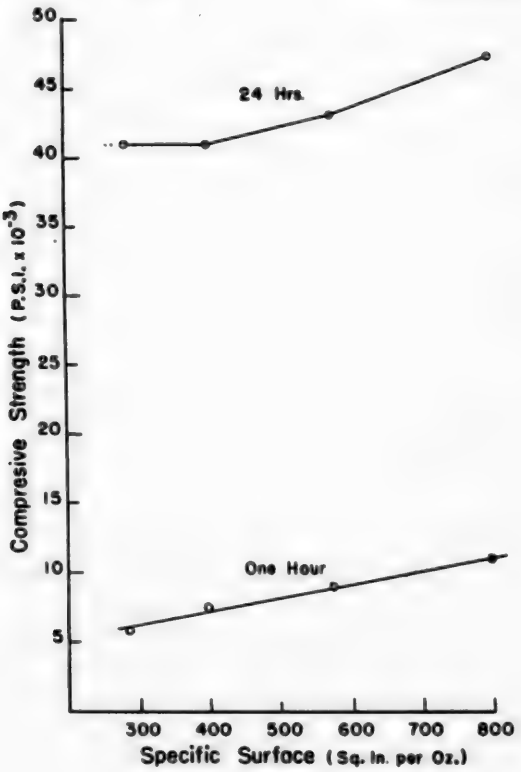


Fig. 4.

Set, as measured by a penetrometer, may be assumed to occur when a constant fraction of the mercury is consumed, i.e., when $\frac{a}{}$ is constant. From equation 3 above, the time at which this occurs is proportional to the reciprocal of the specific surface. Plotting setting time against the reciprocal of the

specific surface should give a straight line. When measuring expansion with the interferometer, measurements begin after the reaction has proceeded for a certain time, which was held constant in this experiment. The magnitude of this expansion can be assumed to be proportional to the unreacted mercury (a-x), remaining in the mass at this time. From equation 4, log (a-x) is proportional to S, the specific surface, and a straight line should be produced.

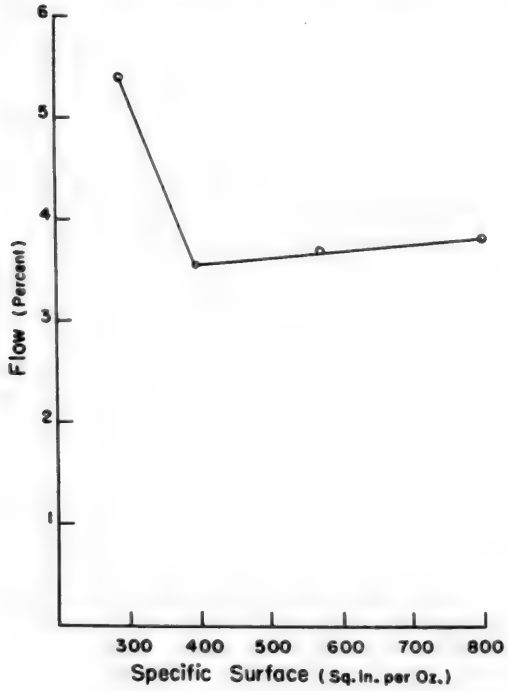


Fig. 5.

As would be expected from the setting time data, the one-hour strength is almost a straight line function of the specific surface as shown in Fig. 4. The more rapid setting high specific surface samples gain their strength much more rapidly than the thicker cuts. In fact, the cut having a specific surface of 801 square inches per ounce had twice the early strength of the thicker particles which had a surface area of only 287 square inches per ounce. The 24-hour compressive strength data (Fig. 4) likewise show a slight increase in strength as the surface area is increased. The increase in strength from the cut having the smallest specific surface to that having the greatest was approximately 12 per cent.

The flow data (Fig. 5) on these composite samples compare favorably with that obtained earlier on center cuts taken from only one area of the ingot (Table II). The flow value for the thick cut having the least surface was considerably higher than the figures obtained for the other three particle sizes.

CONCLUSIONS

The physical properties of an amalgam alloy having particles of varying specific surface have been studied. The specific surface was controlled by varying the thickness of the chip.

Data show that the properties of chips, of a given thickness, cut from different areas of the same mold will vary. When tests were made on cuttings of varying thicknesses from adjoining areas of the ingot, it was found that the expansion is a linear function of the surface area. When the setting time is plotted against the reciprocal of the relative surface area, the curve approaches a straight line.

The greater the surface area, the higher is the compressive strength. The flow remains constant except for the thickest cuttings here tested which, of course, have the lowest specific surface. The flow on this sample was over 1 per cent higher than on the others.

One-hour compressive strength, as might be expected from the setting time data, increases as specific surface increases. Twenty-four hour strength shows a similar trend, although, the per cent difference between the lowest and highest values is only 12 per cent.

The samples of alloy used in this work were prepared in the Research Department of the S. S. White Dental Manufacturing Company. A large number of the expansion and flow measurements were made by Mr. G. W. Birch of the above company, to whom we wish to express our thanks.

REFERENCES

1. Jarabak, J. R.: The Effect of Particle Size on Dimensional Change in Dental Amalgams, *J. A. D. A.* **29**: 593, 1942.
2. Gray, A. W.: Contraction and Expansion of Amalgams With Time, *Phys. Review* **18**: 108, 1921.
3. Gray, A. W.: Volume Changes in Amalgams, *J. Inst. Metals* **29**: 139, 1923.
4. Taylor, N. O., Sweeney, W. T., Mahler, D. B., and Dinger, E. J.: The Effects of Variable Factors on Crushing Strengths of Dental Amalgams, *J. D. Res.* **28**: 228, 1949.
5. Phillips, R. W.: Compressive Strength of Amalgam as Related to Time, *J. D. Res.* **28**: 348, 1949.
6. Murphy, A. J.: The Constitution of the Alloys of Silver and Tin, *J. Inst. Metals* **35**: 107, 1926.

A STUDY OF THE CUTTING EFFICIENCY OF DENTAL BURS FOR THE STRAIGHT HANDPIECE

E. E. HENRY, M.S.E., AND F. A. PEYTON, D.Sc., ANN ARBOR, MICH.

School of Dentistry, University of Michigan

IN THE preparation of cavities through the use of rotating instruments utilizing burs and stones, the ideal condition of design and operation has been described as the one which will remove the most material with the least patient discomfort due to heat, vibration, and pressure, and which also produces the longest life of the operating instrument. Some have recommended new and ingenious methods of material removal. The Air-Dent method is one example,[1] while Walsh and Symmons[2] in New Zealand have advocated the use of a dental bur at a speed of 60,000 r.p.m. Others advocate the use of diamond pointed instruments at high speed. The use of diamond instruments, however, is not recommended for soft materials. According to one investigator, they should not be used against amalgams and metals. This material should be removed by burs.[4]

Studies on some causes of heat and vibration have been conducted and reported.[5, 6] A third study, namely, the amount of material removed or efficiency of the instrument, is in order and a portion of that study is reported at this time.

The amount of material removed is a function of several variables, such as the instrument design, material to be cut, speed, and pressure. The composition of the bur is also a variable, particularly when the active life of the bur is considered. It is therefore necessary at the outset to establish the limits that will be considered in this portion of the study.

The first is the material to be cut. This report will be limited to results obtained through the use of Ivoryene, a synthetic ivory, having a hardness somewhat comparable to dentin. It has the advantage over actual tooth material of being more uniform and homogeneous. A second condition is the active life of the bur. To overcome this variable, each test (except for carbide burs, stones, and diamond points) was conducted with a new bur. Therefore, no consideration of dulling or bur life is taken into account. These conditions approach the ideal in the case of burs, but perhaps are too ideal for everyday use. This practice was necessary, however, in order to form some measure of standardization from which to obtain information on bur design.

EQUIPMENT AND PROCEDURE

A Meisinger dental motor, capable of rotating at a speed of approximately 12,000 r.p.m. when a voltage of 135 volts is applied, was calibrated by the accepted Prony brake method. Using a constant voltage, the speed, obtained by the use of a stroboscope, and the amperage were recorded for each load applied. A calibration graph was plotted, representing the speed against amperage and torque against amperage, for the different increments of 5 volts between 60 volts to 110 volts. A typical graph is shown in Fig. 1.

This study was supported by a research grant (Project D-47C-2) from the National Dental Research Institute, U. S. Public Health Service.

Read at the meeting of the Materials Group, International Association for Dental Research, March 17-18, 1951, French Lick, Ind. (*J. D. Res. 30:* 497, 1951).

Received for publication, June 1, 1951.

The cutting equipment consisted of a four-wheel carriage mounted on tracks, and counterbalanced to overcome frictional forces. A clamp for holding the specimen (Ivoryene block) was fixed in the center of the carriage. To maintain the desired constant feed or pressure, a pan was attached with a fine wire, on which the different weights could be placed.

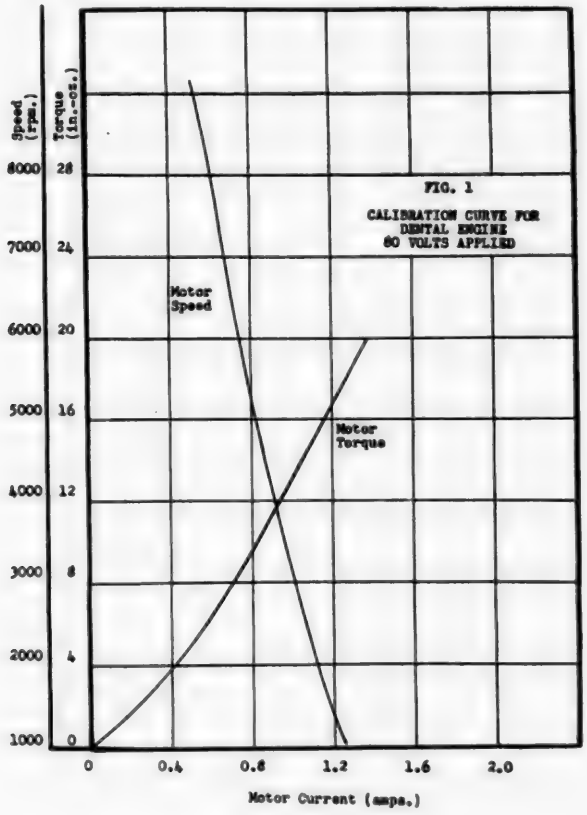


Fig. 1.

The handpiece was firmly held beneath the tracks and could be raised or lowered to insure that the same length of bur was being used for each test. The bur was always placed in the handpiece collet to the same depth and securely held in position to standardize conditions further. As previously noted, a new bur was used for each cutting operation, except in the cases mentioned.

The energy necessary to operate the handpiece alone was determined at various speeds by varying the input voltage of the motor through the full range of calibration. This was followed by the test program (Table I) in which each bur was tested throughout the full speed range for each pressure.

The results of these tests are shown in graphical form in Figures 2 through 14.

TABLE I

BUR TYPE	BUR NO.	PRESSURE (GRAMS)	MFGR.*	REMARKS
Fissure	557	100, 200, 500, 750, 1000	1	Steel, straight flutes.
	557	100, 500	3	Tungsten carbide.
	557	200, 500, 750,	2	Steel, straight flutes.
	557	200, 500, 750,	2	Steel, spiral flutes.
	558	100, 200, 500, 750	1	Steel, straight flutes.
Inverted Cones	37	100, 200, 500, 750	1	Steel.
	37	100, 200, 500, 750	3	Tungsten carbide.
	39	100, 200, 500, 750	1	Steel.

*Products of only 3 bur manufacturers, designated Nos. 1, 2, and 3, were used.

DISCUSSION OF RESULTS

In Fig. 2 is shown a relationship between the material removed in grams per minute and the energy expended for the No. 557 fissure bur. This energy is given as power (energy per unit time) by the ordinate NT where N represents speed in revolutions per minute and T represents the torque in inch-ounces.

The curves are plotted on log-log paper so that they approach and are drawn as straight lines. These curves were drawn visually. For a high degree of accuracy, they should be calculated by the method of least squares. However, because of the expenditure of time involved in comparison with the information obtained, this was not done.

There are two points of importance to be considered on this type of graphical ploto. The first is the relative position of one particular curve with respect to the other curves, and the second is the slope of each curve. A curve for one bur which is to the right of the curve for another bur would mean that more material is removed for the same expenditure of energy by the bur on the right hand side. The slope of the curve denotes the rate of increase of material removal with respect to an increase of energy expended.

For a light load (100 Gm.-0.22 pounds), the tungsten carbide bur is shown to be superior to the ordinary steel bur (No. 557 fissure type bur). This is shown by the curve for the carbide bur in Fig. 2 which is considerably to the right of that of the steel bur.

With a two-hundred gram load applied, the same bur (No. 557) was compared[1] with a similar bur made by another producer, and with[2] another type No. 557 bur on which the axial cuts are spiral instead of straight.

At a load of 500 grams (1.1 pounds), the bur with spiral axial grooves is shown to be slightly superior to the bur with straight grooves, as indicated by the horizontal spacing between curves. The same tendency is shown by the curves obtained at a load of 750 grams. It is interesting to observe that, on an energy basis, a rather large increase in material removal (grams per minute) is achieved when the pressure is raised from 200 grams to 500 grams; whereas, when the pressure is increased from 500 grams to 750 grams, the amount of material removed is increased, but the relative increase is considerably less than that between the 200 and 500 gram load change. This, however, is on an energy

basis, which is purely physical and should not be construed to mean that from a standpoint of dental operating efficiency the same conclusion would hold. The amount of material removed per unit time with changes in speed and pressure will be presented later in this report.

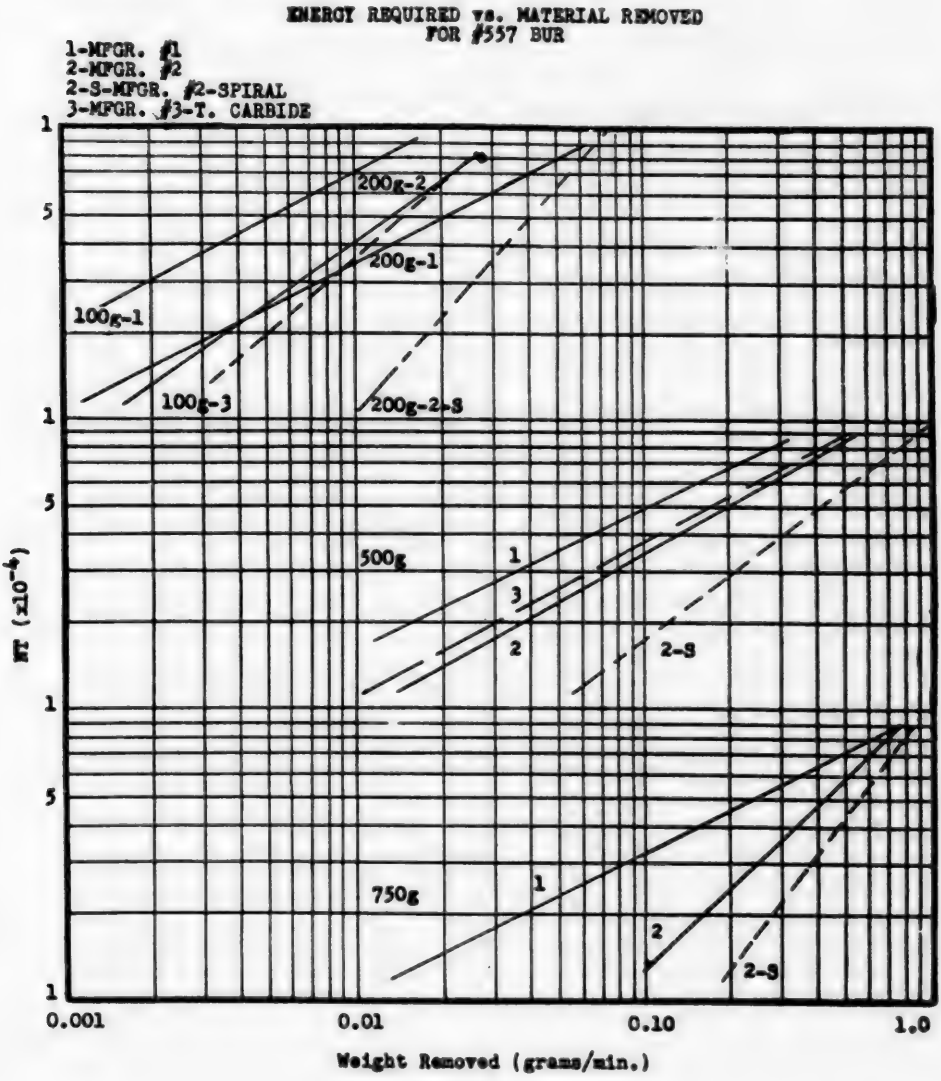


Fig. 2.

In Figs. 3-6 are shown the amounts of material removed as a function of the speed of the motor, for pressures of 100 grams (0.22 pounds), 200 grams (0.44 pounds), 500 grams (1.1 pounds), and 750 grams (1.65 pounds), respectively. On each curve is shown a vertical line at 4000 r.p.m. This is considered to be

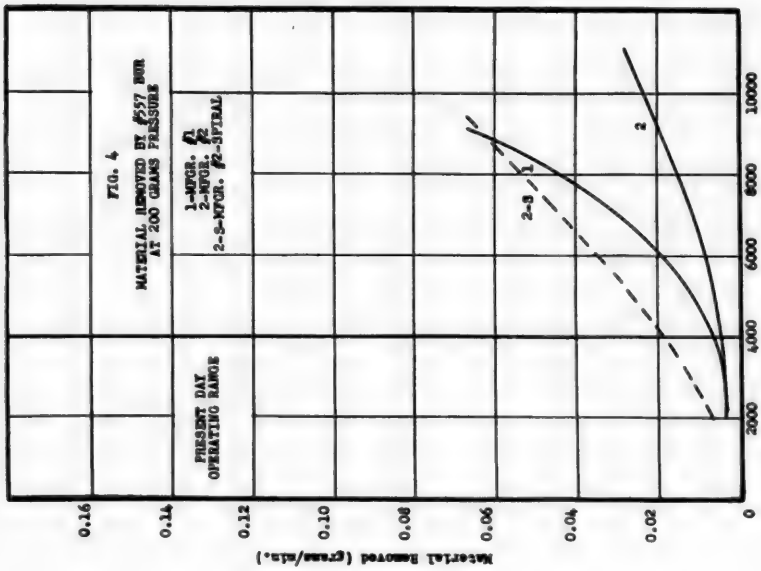


FIG. 4
MATERIAL REMOVED BY #557 SUB AT 200 GRAMS PRESSURE
1-MTGR. #1
2-MTGR. #2
2-3-MTGR. #2-SPIRAL
PRESENT DAY OPERATING RANGE
Material Removed (grams/min.)
Speed (rpm.)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
2000
4000
6000
8000
10000
1
2
2-3

Fig. 4.

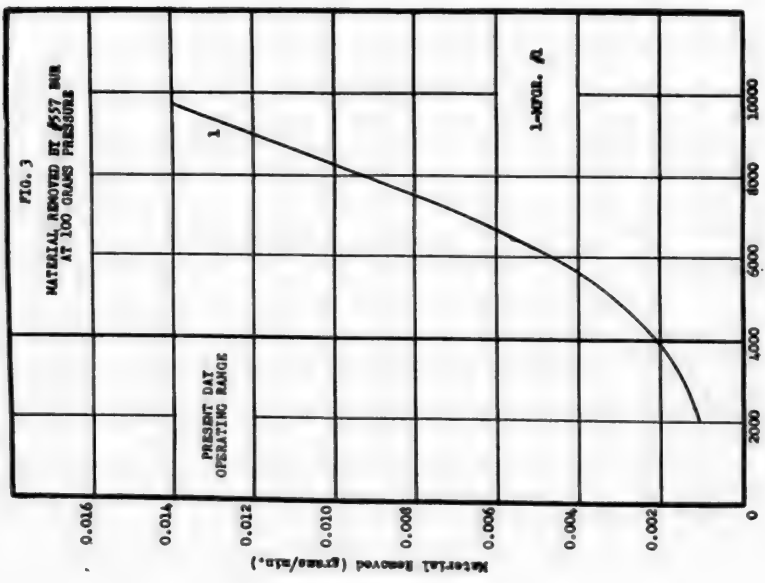


FIG. 3
MATERIAL REMOVED BY #557 SUB AT 100 GRAMS PRESSURE
1-MTGR. #1
PRESENT DAY OPERATING RANGE
Material Removed (grams/min.)
Speed (rpm.)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0
2000
4000
6000
8000
10000
1

Fig. 3.

close to the average maximum limit of the present day operating range, since most dental engines have a maximum speed of 3600 r.p.m., while others approach 4500 r.p.m. These curves are all of the same general shape and show a large increase in the material removed as the speed is increased beyond 4000 r.p.m.

The curves in Fig. 5 show that the fissure bur (No. 557), made of tungsten carbide, and the two different No. 557 steel burs by different manufacturers, all with straight cut axial grooves, produce essentially the same performance. The steel bur with spiral cut axial grooves was superior to those with straight grooves, except possibly at high speeds (in the order of 10,000 r.p.m.). Observations when the tests were carried out would indicate that the No. 557 spiral burs reach a maximum cutting ability at about 9000 r.p.m., and beyond that speed the amount of material removed per unit time decreases. This condition could be due to a filling of the flutes of the bur with Ivoryene, which is relatively soft, and also being a plastic may further soften with elevated temperature. This tendency of the amount of material removed to decrease at high speeds is less pronounced as the load is increased, as shown in Fig. 6.

The data on steel burs from these curves (Figs. 3-6) are condensed in Table II.

TABLE II

BUR MFGR.	PRESSURE (GRAMS)	WEIGHT (GRAMS PER MINUTE) REMOVED AT SPEEDS OF			
		2000 RPM.	4000 RPM.	6000 RPM.	8000 RPM.
No. 557 1	100	.0011	.0022	.0047	.0097
	200	.003	.007	.019	.044
	500	.015	.067	.17	.32
	750	.08	.19	.32	.495
No. 557 2 Straight Flutes.	200	.003	.005	.0085	.015
	500	.03	.085	.18	.31
	750				
No. 557 2 Spiral Flutes.	200	.007	.028	.034	.053
	500	.07	.225	.40	.487
	750				

One general rule that may be concluded from the data is that for the straight grooved No. 557 bur, the amount of material removed in grams per minute is approximately doubled for each speed increase of 2000 r.p.m. up to 8000 r.p.m. while the applied pressure remains constant.

An indication of the amount of material removed as a function of the speed and pressure applied is shown in the three dimensional Fig. 7. This is shown as a surface whose vertical ordinate is the material removed. The line of intersection of a horizontal plane (an imaginary plane parallel to the base plane) with the surface would show the different combinations of speed and pressure that would remove a given amount of material per minute. The amount of material removed is represented by the vertical height at which the plane is drawn.

CHARACTERISTICS OF NO. 558 FISSURE BUR

In Fig. 8 is shown the relationship between the amount of material removed per minute and the energy input for a No. 558 bur at different pressures. This

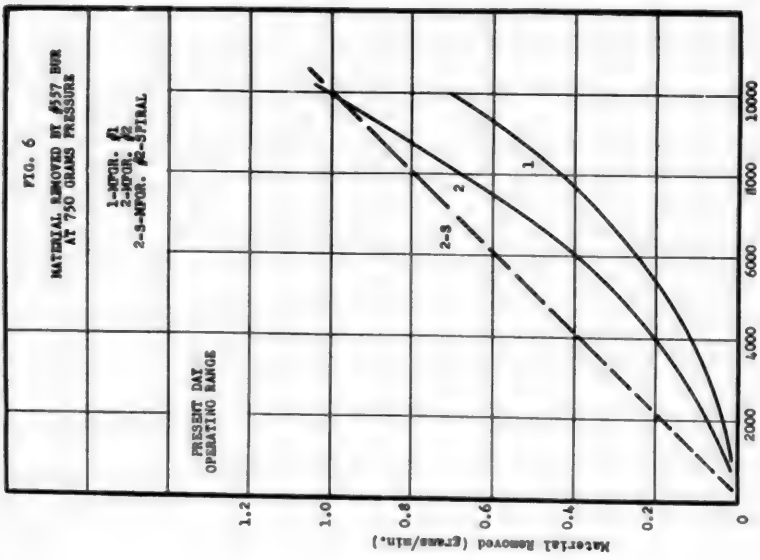


FIG. 6
MATERIAL REMOVED BY #557 BUR AT 750 GRAMS PRESSURE
1-NPGR. #1
2-NPGR. #2
2-3-NPGR. #2-SPIRAL
PRESENT DAY OPERATING RANGE
Material Removed (Grams/min.)
Speed (rpm.)
0
0.2
0.4
0.6
0.8
1.0
1.2
2000
4000
6000
8000
10000
1
2
2-3

Fig. 6.

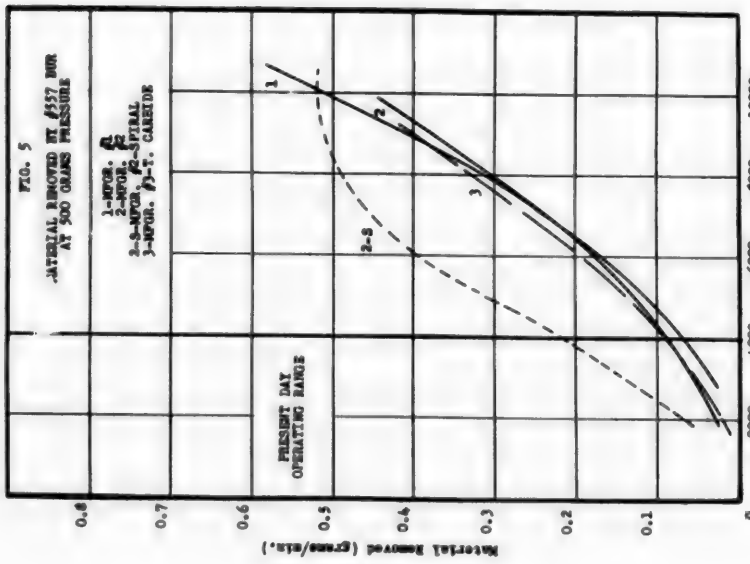


FIG. 5
MATERIAL REMOVED BY #557 BUR AT 500 GRAMS PRESSURE
1-NPGR. #1
2-NPGR. #2
2-3-NPGR. #2-SPIRAL
3-NPGR. #3-T. CARETOR
PRESENT DAY OPERATING RANGE
Material Removed (Grams/min.)
Speed (rpm.)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
2000
4000
6000
8000
10000
1
2
3
2-3

Fig. 5.

TABLE III

PRESSURE (GRAMS)	MFGR.	AMOUNT (GRAMS/MINUTE) REMOVED AT SPEEDS OF			
		2000 RPM.	4000 RPM.	6000 RPM.	8000 RPM.
100	1	.0014	.0033	.0059	.0117
200		.003	.01	.02	.034
500		.03	.062	.105	.17
750		.06	.155	.292	.61

is similar to Fig. 2 for the No. 557 fissure bur. As was true for the No. 557 bur, considerably more material is removed by the No. 558 bur when the pressure is raised from 200 grams to 500 grams, than from 500 grams to 750 grams. For a No. 558 bur the amount of material removed as a function of speed and pressure is shown in Table III.

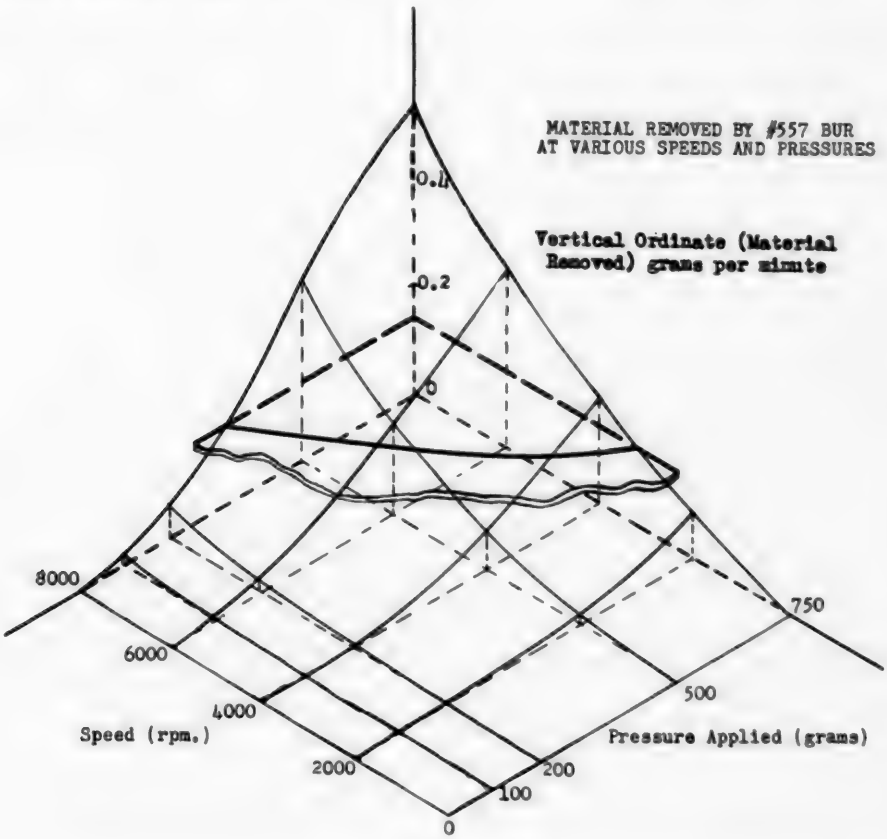


Fig. 7.

These data show that the amount of material removed per minute by a No. 558 fissure bur is not greatly different from that removed by the No. 557 bur. In some cases, the No. 558 bur removes more material, as one would feel it

should, since it is a larger bur. This is particularly true at a lighter load of 100 grams. It is also true at a load of 750 grams and 8000 r.p.m. However, the differences in any situation are not large.

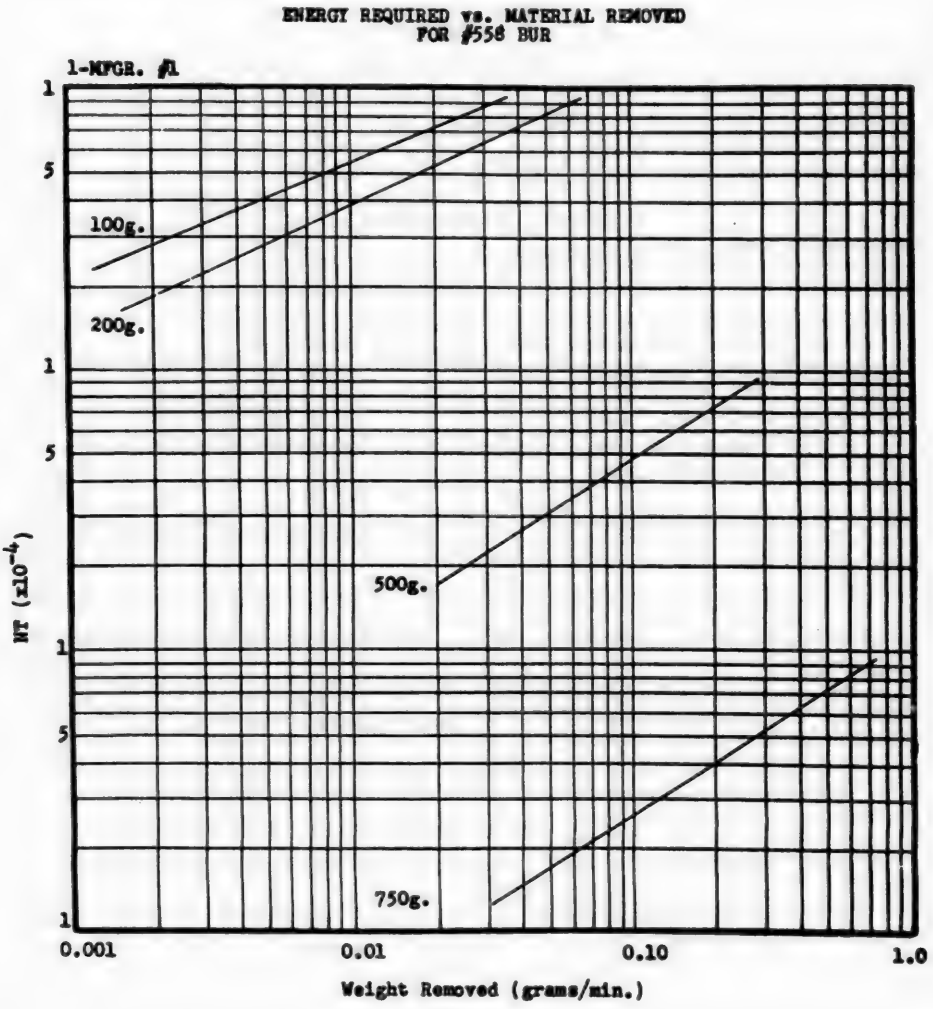


Fig. 8.

In Fig. 9, which is comparable to Fig. 7, is shown the material removed as a function of both speed and pressure. It was observed in using the No. 557 and No. 558 burs that the material which had been cut away was packed rather hard in the cut. If this is characteristic of this material or type of cut only, it would be of no practical consequence, but if this should happen in actual cavity preparation, it might have an influence on the problem of heat generation. This effect did not occur with the inverted cone type bur, which is described presently.

CHARACTERISTICS OF THE NO. 37 INVERTED CONE BUR

The No. 37 inverted cone bur has an average diameter of approximately 0.045 inch and a length of 0.050 inch, while the No. 557 fissure bur has an average diameter of 0.038 inch and a length of 0.185 inch. A notable difference between the No. 37 and No. 39, and the No. 557 and No. 558 is that although the inverted cone type bur possesses about one-third the active cutting area of the fissure type bur, it is capable of removing approximately five times as much material per unit time. This is demonstrated by comparing the abscissa coordinated (material removed-grams per minute) which for the No. 37 inverted cone burs (Fig. 10) is ten times as great as those for the No. 557 fissure bur previously shown in Fig. 2. This observation should be somewhat tempered

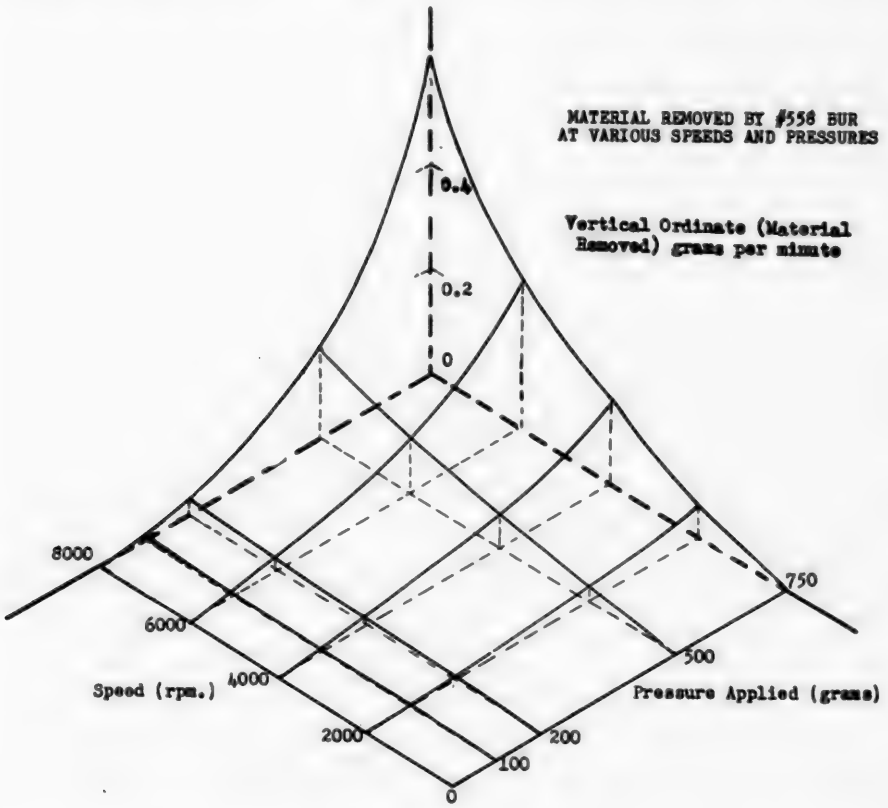


Fig. 9.

though by noticing that the curves are not similarly placed. As an example, with an input energy (NT) of 30,000 (.03HP), and a pressure of 750 grams, an inverted cone No. 37 bur removed 0.62 grams per minute while a No. 557 fissure bur removed 0.08 grams per minute, and a No. 558 fissure bur, 0.12 grams per minute.

TABLE IV

PRESSURE (GRAMS)	MFGR.	MATERIAL (GRAM/MINUTE) REMOVED AT SPEEDS OF			
		2000 RPM.	4000 RPM.	6000 RPM.	8000 RPM.
100	1	.001	.007	.026	.075
200		.02	.074	.15	.24
500		.21	.44	.72	1.02

The amount of material removed as a function of both speed and pressure is given in Fig. 11. The data in Table IV were used to construct Fig. 12.

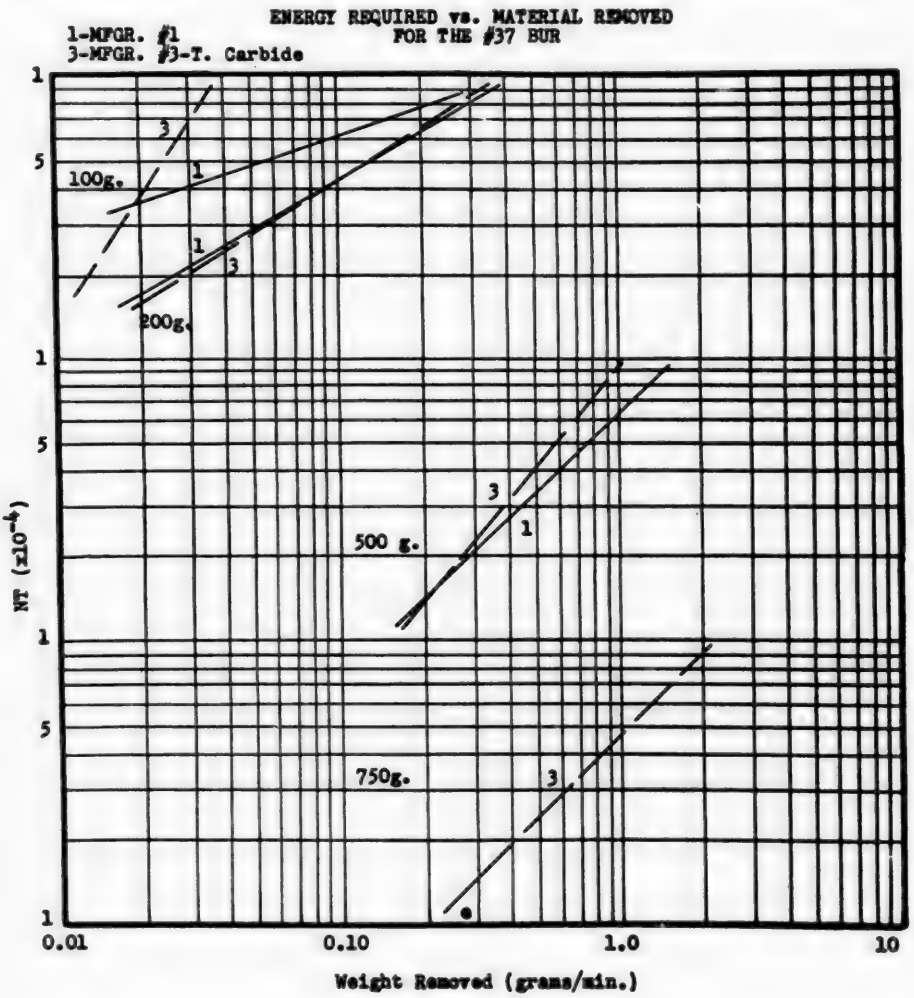


Fig. 10.

CHARACTERISTICS OF THE NO. 39 INVERTED CONE BUR

The relationship between the material removed per minute and the energy input for pressures of 100 grams, 200 grams, 500 grams, and 750 grams is given

in Fig. 13. There is a considerable difference in the amount of material removed when the applied pressure is varied. For example, with an energy input (NT) of 30,000 the following Table V is taken from this graph.

TABLE V

PRESSURE (GRAMS)	MATERIAL REMOVED (GRAMS PER MINUTE)
100	.005
200	.092
500	.400
750	.720

With an increase from 100 to 200 grams, the amount of material increased 0.087 grams per minute. With an increase from 200 to 500 grams pressure, or an increase of 300 grams, there was an increase of 0.31 grams per minute, which is an average of 0.10 grams per minute increase per 100 grams increase of load. From 500 to 750 grams pressure, the increase in material removal was 0.31 grams per minute, which would be an increase of 0.13 grams per minute per 100 grams increase in pressure. This indicates that as the pressure was raised more material was removed on a constant input energy basis, but takes into account only the kinetic energy of the motor, and leaves out any consideration of the potential energy due to the pressure exerted by the operator.

The amount of material removed as a function of speed and pressure is shown by Table VI.

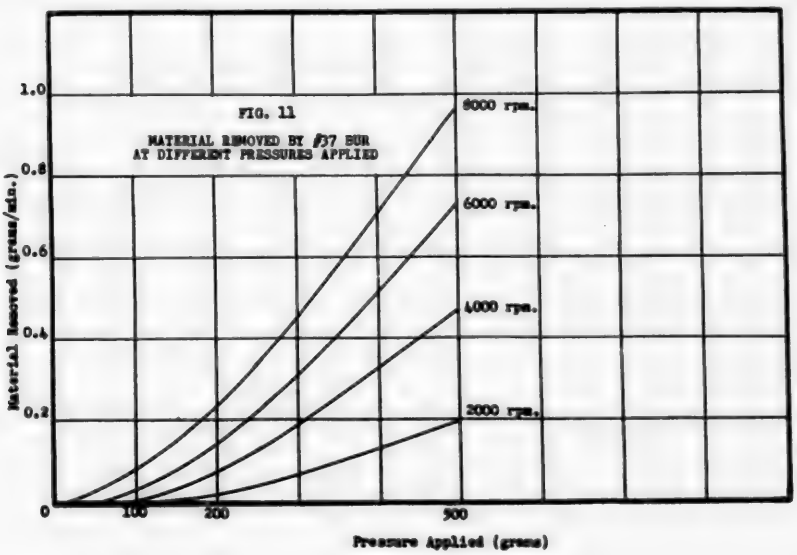


Fig. 11.

This table is plotted as a surface in Fig. 14 and it should be realized that the surface extends beyond the limits of this study.

TABLE VI

PRESSURE (GRAMS)	MATERIAL (GRAMS PER MINUTE) REMOVED AT SPEEDS OF			
	2000 RPM.	4000 RPM.	6000 RPM.	8000 RPM.
100	.007	.016	.0295	.054
200	.027	.098	.17	.242
500	.28	.56	.84	1.12
750	.34	.84	1.51	3.4

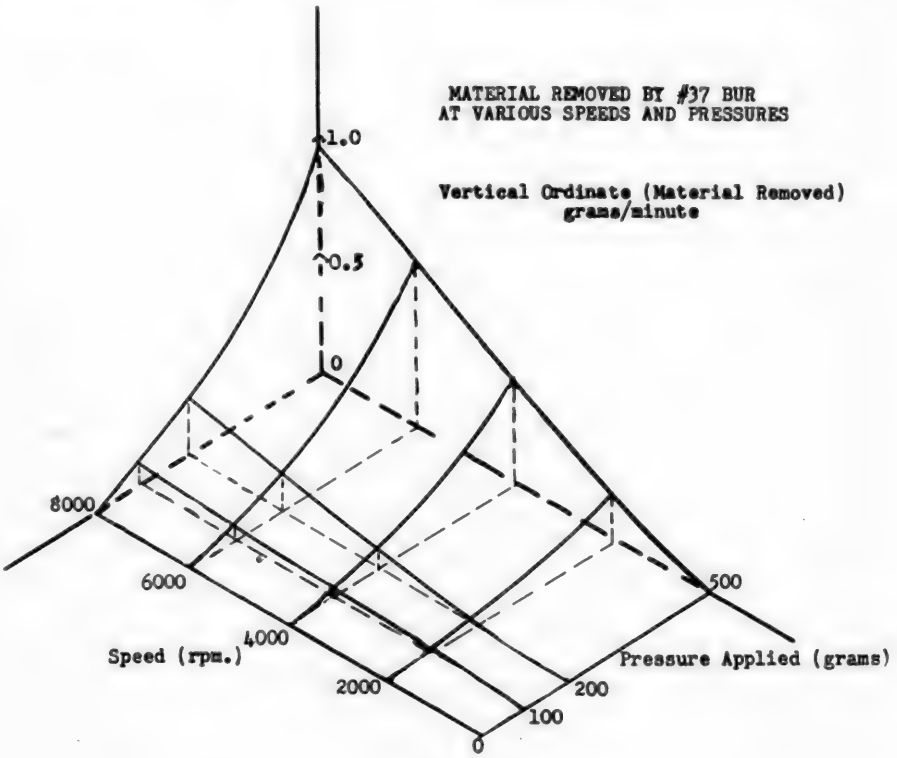


Fig. 12.

SUMMARY

This study has been limited to a comparison of the efficiency of the fissure and inverted cone type of dental burs when cutting a material with a hardness similar to dentin. This permitted a comparison of bur types on a basis of design only and did not consider the endurance of the bur, or the tendency to become dull which would result if hard materials like glass or enamel were cut. The effect of bur size and composition as related to cutting efficiency were also studied to a limited extent. The physical factors of speed of rotation between the limits of 1000 and 8000 r.p.m., and force applied to the cutting instrument from 200 grams (0.44 pounds) to 750 grams (1.65 pounds) were studied. An effort was made to integrate these variables as far as practicable.

It was observed that the tungsten carbide burs examined cut at essentially the same rate as the steel burs when operated under comparable conditions, but the carbide burs retain their cutting edge longer. The carbide burs were more brittle and susceptible to breakage than steel burs when subjected to side pressure and torque due to being embedded in the material cut.

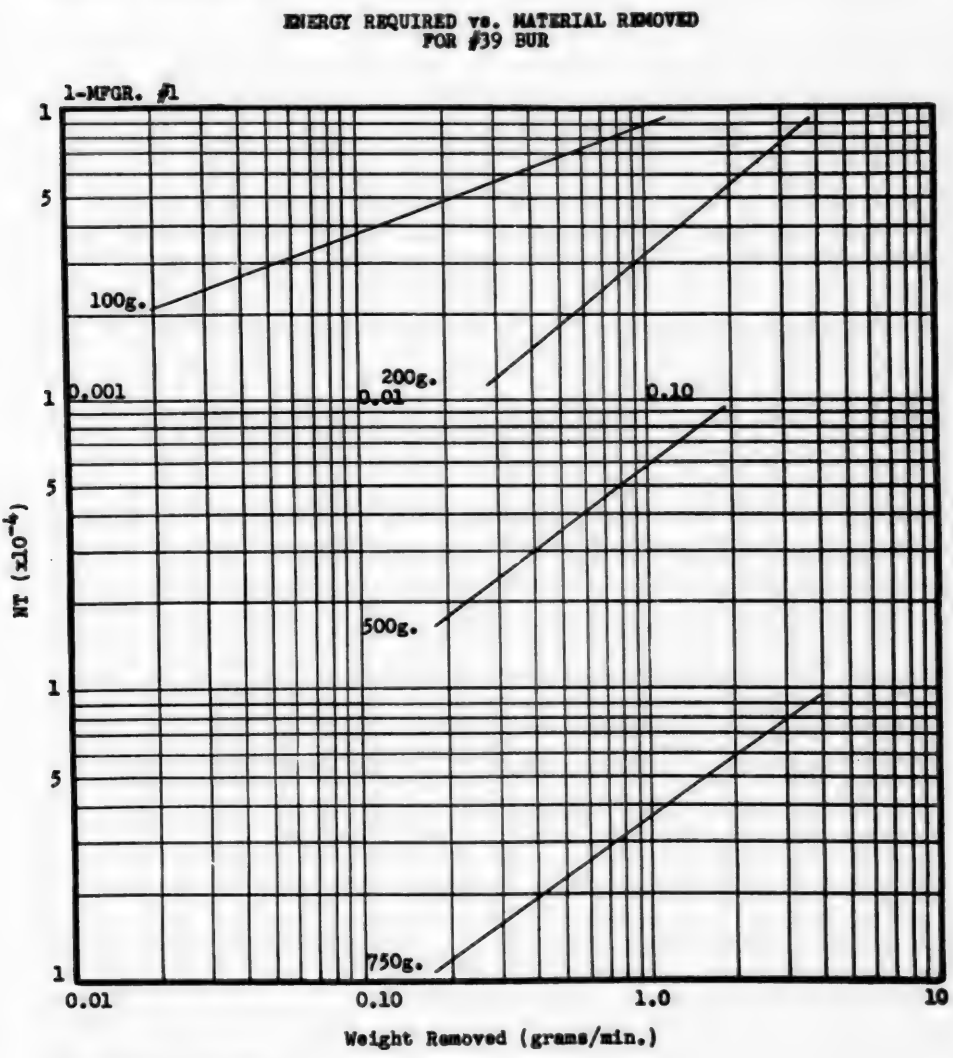


Fig. 13.

The No. 557 fissure type steel bur with spiral axial grooves removed more material per unit time than burs with straight grooves when operated under the same conditions. It was observed that the spiral type bur showed a little greater tendency to become clogged with cut material than did the straight bur.

A pronounced difference in the amount of material removed per unit time by the inverted cone type bur as compared with the fissure type bur was observed. Although the No. 37 bur has only about one-third as much active area as the No. 557 fissure bur, it is capable of removing approximately five times the amount of material per unit time as the fissure bur. On a basis of unit active area this would indicate a ratio of approximately 15 to 1 in favor of the inverted cone type.

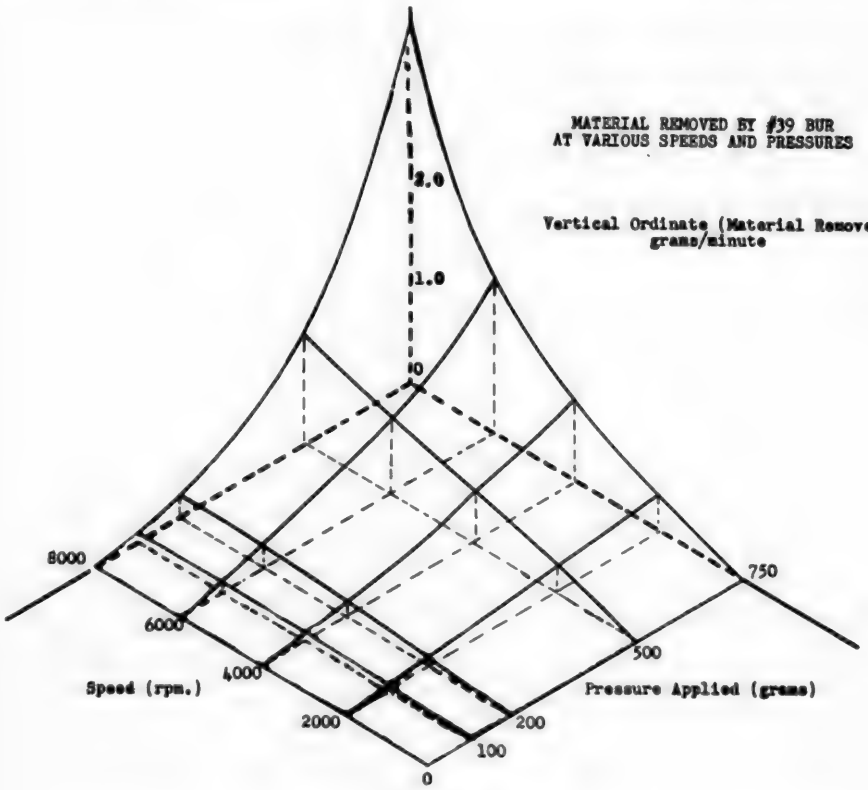


Fig. 14.

When using the No. 557 steel bur it was found that for each speed increase of 2000 r.p.m., between the limits of 2000 and 8000 r.p.m., the amount of material removed per unit time was approximately doubled, while the applied pressure remained constant. Accordingly, the rate of removal increases rapidly at speeds greater than 4000 r.p.m., which is near the upper limit of most present day engines.

When using a No. 557 bur with a constant energy input, a comparatively large increase in material removed is achieved when the pressure is raised from 200 to 500 grams. With an increase in pressure from 500 to 750 grams, the

amount of material removed is increased, but the relative increase is less pronounced. With an inverted cone bur, however, this trend is reversed so that with increased pressure there is an increased rate of removal of material.

The results reported indicate that an increase in size of bur for a given type will result in greater amounts of material removed when other factors remain constant.

REFERENCES

1. Black, R. B.: Technic for Nonmechanical Preparation of Cavities and Prophylaxis, *J. A. D. A.* **32**: 955, 1945.
   Black, R. B.: Airbrasive: Some Fundamentals, *J. A. D. A.* **41**: 701, 1950.
2. Walsh, J. P., and Symmons, H. F.: Vibration Perception and Frequencies, *New Zealand D. J.* **45**: 106, 1949.
3. Larson, N. H.: The Efficient Use of Carbide Burs and Diamond Points for Cavity Preparations, *D. Dig.* **55**: 442 and 495, 1949.
4. Hislop, T., and Hislop, R. B.: Notes on Diamond Instruments, *D. Mag. & Oral Topics* **67**: 268, 1950.
5. Peyton, F. A., and Vaughn, R. C.: Thermal Changes Developed During the Cutting of Tooth Tissue, *Chicago D. Soc. Fortn. Rev.* **20**: 9, 1950.
6. Henry, E. E., and Peyton, F. A.: Vibration Characteristics of the Rotating Dental Instrument, *J. D. Res.* **29**: 601, 1950.

LEVO-ARTERENOL (LEVOPHED) AS A VASOCONSTRICTOR IN LOCAL ANESTHETIC SOLUTIONS

SIDNEY EPSTEIN, D.D.S., ALBERT H. THRONDSON, D.D.S., AND J. LEONARD SCHMITZ, D.D.S.

College of Physicians and Surgeons, School of Dentistry, San Francisco, California

A NUMBER of vasoconstrictors have been introduced from time to time as substitutes for epinephrine in dental local anesthetic solutions. There has been a clinical impression that several of these, particularly nordefrin (Cobefrin) and phenylephrine hydrochloride (Neo-Synephrine) are safer agents than epinephrine, since their use has been attended by fewer reactions or toxic manifestations. Whether this impression is correct or not, the fact still remains that the ideal vasoconstrictor agent has not yet been found, although those now in use are highly satisfactory in most respects. Accordingly, when Tainter, Tullar and Luduena[1] succeeded in resolving arterenol into its optical isomers, it appeared desirable to evaluate the levo form of this compound (available under the trademark name of Levophed) as a component of local anesthetic solutions. This compound appeared to be of special interest in view of the fact that toxicity figures indicate that for equivalent pressor doses, the safety of levoarterenol is approximately four times that of levo-epinephrine.

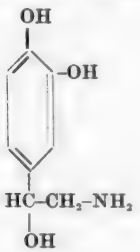
The pharmacology of arterenol has been summarized by Luduena, Ananenko, Siegmund, and Miller[2] and need not be reviewed here; however, it is of interest to note that arterenol is a hormone occurring naturally in the body. It has been isolated in chemically pure form from adrenal medulla extracts and has been shown to be present in tumors of the adrenal medulla, along with epinephrine. It is secreted by the normal adrenal gland in response to reflex stimulation, is present in sympathetic nerves, and is released from the nerve trunks in vitro during stimulation.[3] It appears that arterenol, perhaps along with epinephrine, may be a hormone involved in the chemical transmission of peripheral nerve impulses in the sympathetic nervous system. It has long been suspected that this hormone, if it could be isolated, might be preferable to epinephrine as the vasoconstrictor in local anesthetic solutions, since it is almost completely free of the vasodilator effects seen with epinephrine and which have been suspected of sometimes being implicated in postoperative hemorrhage.

Dobbs[4] has published a preliminary report on the effectiveness of levo-arterenol as a vasoconstrictor in local anesthesia. As a result of his work, Dobbs concluded that a concentration of levo-arterenol greater than 1:50,000, and less than 1:30,000, will give comparable vasoconstriction to nordefrin 1:10,000 when combined in a similar anesthetic mixture. In the present study, levo-arterenol was employed at a concentration level of 1:30,000 in two different local anesthetic bases: (1) in combination with procaine 2 per cent, and (2) in combination with procaine (Novocaine) 2 per cent with tetracaine (Pontocaine) 0.15 per cent. Two other solutions were used for purposes of comparison: (3) epinephrine 1:50,000 with procaine 2 per cent, and (4) nordefrin 1:10,000 with procaine

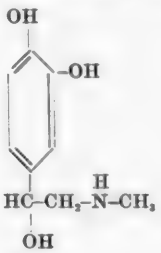
Received for publication, April 24, 1951.

2 per cent and tetracaine 0.15 per cent. The results obtained with solutions (1) and (3) will enable one to compare levo-arterenol with L-epinephrine, while the results obtained with solutions (2) and (4) make it possible to compare arterenol with nordefrin. The chemical relationship between arterenol, epinephrine, and nordefrin can be seen from the structural formulas in Fig. 1.

Epinephrine can be considered as an N-methylated derivative of arterenol, while nordefrin is a C-methylated derivative of arterenol.



Levo-arterenol



Epinephrine

Nordefrin

The observations recorded in this paper were made entirely on private office patients, subjected to the ordinary operative procedures of oral surgery and operative dentistry, under local anesthesia. The familiar "blind test" methods developed by Tainter[5] were used throughout the study as in a recently reported similar investigation.[6] The four solutions were supplied in cartridge form, identified only by code numbers, so that the operators were unaware at all times of the identity of the solutions used.

RESULTS

A breakdown was made according to type of operation, i.e., dental surgery or operative dentistry. This was done because some investigators believe that operative dentistry requires a more profound anesthesia than does exodontia or dental surgery. Unfortunately, there was such a preponderance of patients in the surgical series (659 vs. 98 operative) that one is not justified in comparing the results of the two types of procedures. It is interesting to note that in the case of two of the solutions, a higher percentage of adequate anesthesia was obtained in surgical operations than in operative. In the case of the other two solutions, the reverse holds true. However, as before stated, since there were not enough patients in the operative series, the apparent differences cannot be considered conclusive. Accordingly, in Table I, the surgery and operative patients for each solution were combined into a single figure, so that the results on both operative and surgery patients can be compared as a unit for each of the four solutions. A total of 757 patients were studied in this series, or an average of 189 patients per solution. This number is sufficiently large that even relatively small differences in the average results may indicate true differences between the solutions, rather than chance variations in the patients.

From Table I it will be seen that the distribution according to the site of injection is approximately the same for the four solutions. Therefore, the types

TABLE I

COMBINED DATA (DENTAL SURGERY

SOLUTION	OPERATION	NO. OF PATIENTS	SITE OF INJECTION			
			INFILTRATION	MANDIBULAR	TUBEROSITY	INFRAORBITAL
2% procaine 1:50,000 epinephrine	Combined	209	63 25.81 ±2.8	108 44.26 ±3.2	68 27.86 ±2.9	5 2.04 ±0.9
2% procaine 0.15% tetracaine 1:10,000 nordefrin	Combined	236	74 25.87 ±2.6	136 47.55 ±2.9	62 21.67 ±2.4	14 4.89 ±1.3
2% procaine 1:30,000 arterenol	Combined	167	41 20.81 ±2.9	80 40.60 ±3.5	63 31.97 ±3.3	13 6.59 ±1.8
2% procaine 0.15% tetracaine 1:30,000 arterenol	Combined	145	40 25.64 ±3.5	58 37.17 ±3.9	54 34.61 ±3.8	4 2.56 ±1.2

of dental procedures used with each solution were sufficiently alike that any differences observed could be ascribed to differences in the solutions.

If one were to use adequacy of anesthesia as the sole criterion, it is apparent that the solution containing levo-arterenol 1:30,000, procaine 2 per cent and tetracaine 0.15 per cent is the solution of choice. Adequate anesthesia was obtained in 97.88 per cent of the patients, as compared to 92.79 per cent in the corresponding solution containing nordefrin 1:10,000 as the vasoconstrictor. The standard errors for the two figures indicate definitely that this difference between the two solutions is truly significant, and not the result of chance variation. The second choice, when adequacy of anesthesia alone is considered, is that solution containing arterenol 1:30,000 with procaine 2 per cent. The figure for adequate anesthesia is about 5 per cent higher than the corresponding solution containing epinephrine 1:50,000 with procaine 2 per cent. Here again, the standard error would indicate that the differences are real. Accordingly, there seems to be little room for doubt that arterenol 1:30,000 can be expected to give adequate anesthesia in a higher percentage of cases than either epinephrine or nordefrin, all other things being equal.

Adequacy of anesthesia is not the only criterion which should be used in judging local anesthetic solutions. The number and intensity of undesirable side effects are also important. When one examines the column headed "no reaction" it is seen that the solution containing nordefrin 1:10,000 with procaine 2 per cent and tutocaine 0.15 per cent had the lowest incidence. The solution containing epinephrine 1:50,000 with procaine 2 per cent is next in line, when judged by this criterion. The standard error would indicate that the differences between the two solutions are probably not significant. Both solutions containing arterenol gave a slightly higher percentage of side effects than did either of the solutions containing epinephrine or nordefrin. However, the differences here are small enough to be borderline in significance. In none of the cases were the reactions recorded severe enough to cause any concern or diminution of satisfaction with the solution in question.

ANESTHESIA		RESULTS							
							BLEEDING		
EQUATE	INADEQUATE	NO REACTION	PERSPIRATION	TORY EMBARRASSMENT RESPIRA-	NERVOUSNESS	TREMORS	NORMAL	EXCESSIVE	
191	17	195	6	2	9	5	200	9	No.
91.82 ±1.9	8.17 ±1.9	93.30 ±1.7	2.87 ±1.1	0.956 ±0.6	4.31 ±1.4	2.39 ±1.0	95.69 ±1.4	4.31 ±1.4	% ±s.e.
219	17	223	4	1	10	1	202	29	No.
92.79 ±1.5	7.21 ±1.5	94.49 ±1.4	1.69 ±0.8	0.42 ±0.4	4.24 ±1.3	0.42 ±0.4	87.44 ±2.1	12.56 ±2.1	% ±s.e.
161	6	155	7	2	7	5	159	8	No.
96.40 ±1.3	3.60 ±1.3	92.81 ±2.0	4.19 ±1.5	1.20 ±0.8	4.19 ±1.5	2.99 ±1.3	95.20 ±1.6	4.80 ±1.6	% ±s.e.
139	3	132	6	3	10	2	136	8	No.
97.88 ±1.2	2.12 ±1.2	91.03 ±2.4	4.14 ±1.6	2.07 ±1.1	6.90 ±2.0	1.38 ±0.9	94.44 ±1.8	5.56 ±1.8	% ±s.e.

Although the solution containing nordefrin gave fewest undesirable side effects it resulted in a higher incidence of excessive bleeding than did any of the other three solutions. This difference is large enough to be significant. In the case of the other three solutions, there was no significant difference in the incidence of excessive bleeding at the site of the operation, the concentration of arterenol selected having almost exactly the same degree of effect in controlling the vascular bed in the area of injection as did the epinephrine. Therefore, the two compounds were being compared in entirely equivalent doses so far as their vasoconstrictor action was concerned. This gives added importance to the significance of the other differences set forth above.

SUMMARY

Solutions containing arterenol gave adequate anesthesia in a higher percentage of patients than did solutions containing either epinephrine or nordefrin in the concentrations used in this study. However, this higher incidence of adequate anesthesia was obtained to some degree, at least, at the expense of a possibly higher incidence of side effects. This study definitely indicates that arterenol is a vasoconstrictor of considerable merit, and can undoubtedly take its place along with epinephrine and nordefrin as a vasconstrictor suitable for local dental anesthetics. When all the various criteria of a good vasoconstrictor are considered at once, the superiority of arterenol over epinephrine and nordefrin is not universal for all phases of its action.

The authors wish to acknowledge the valued assistance of Dr. M. L. Tainter in drafting this report.

REFERENCES

1. Tainter, M. L., Tullar, B. F., and Luduena, F. P.: *Science* **107**: 39, 1948.
2. Luduena, F. P., Ananenko, E., Siegmund, O. H., and Miller, L. C.: *J. Pharmacol. & Exper. Therap.* **95**: 155, 1949.
3. Tainter, M. L., and Luduena, F. P.: *Recent Progress in Hormone Research* **5**: 3, 1950.
4. Dobbs, E. C.: *J. A. D. A* **40**: 433, 1950
5. Tainter, M. L., Throndson, A. H., and Moose, S. M.: *J. A. D. A.* **24**: 376, 1937.
6. Epstein, S., Throndson, A. H., and Schmidt, J. L.: *J. A. D. A.* **42**: 414, 1951.

FACTORS AFFECTING MASTICATORY PERFORMANCE AND EFFICIENCY AMONG YOUNG ADULTS

R. S. MANLY

Laboratory for Oral Physiology, Tufts College Dental School, Boston, Mass.

SEVERAL investigators have studied masticatory efficiency with the intention of discovering the factors which have important influence on this function. Schutz[1] assumed that the value of a tooth in mastication is dependent upon its occlusal surface and upon the force which carries the tooth against its antagonist and that the surface is most important. In one of his experiments, a subject was fitted with a large upper partial denture from which individual teeth could be removed successively. The results of chewing tests suggested that the first and second molars were about equivalent in importance for mastication and were twice as effective as were the premolars. A similar rating of importance for the teeth was obtained when their occlusal area was read with carbon paper. Furthermore the chewing ability of two subjects was compared before and after raising their bites with an onlay appliance; both occlusal area and masticatory ability was diminished by the appliance.

Dahlberg[2] assumed that the number of occlusal contacts was an important determinant of chewing ability and demonstrated that the number of occlusal contacts is closely related to masticatory area which is obtained from photographic silhouettes of registrations taken with wax strips. The importance of masticatory area was not checked by direct comparison with chewing ability of subjects.

Studies from this laboratory have shown that the masticatory performance of young adults is well correlated with food platform area, the fraction of occlusal area that is measured by light transmission through tooth imprints in a soft, semiopaque strip of wax.[3] This relation is valid when the chewing ability and area are considered separately for right and left halves of the dentition.

The test for performance depended on the weights of chewed peanuts which pass and remain on a 10 mesh screen.[4] Both of the test procedures have been modified recently. The masticatory performance tests are now made on a volumetric rather than upon a gravimetric basis.[5] The wax employed for the area test has been modified in composition to make it soft enough to be used without preheating, and a thicker plastic insert has been used between wax sheets in order to strengthen the strip.

These modifications changed the tests enough to make it advisable to check whether the correlation still existed between masticatory performance and food platform area. From the same study it was possible to discover the mathematical relationship between these two measurements and to search for other potential influences on masticatory performance, such as the number and type of teeth in occlusion, and the dimensions of occlusal portions of the dentition.

Supported by contract N7onr-463, 180-396, Task Order No. 2 of the Office of Naval Research.

Presented at the meeting of the 29th International Association for Dental Research, March, 1951, French Lick Springs, Indiana (*J. D. Res.* **30**: 455, 1951).

Received for publication June 23, 1951.

PROCEDURE

One hundred three freshman and sophomore dental students were employed as subjects for this investigation. Their masticatory performance with peanuts and carrots were determined in duplicate by a published method[5] for both right and left halves of their dentition. Food platform area was recorded in duplicate using a new wax composition to be described later. The masticatory efficiency[4] for 54 half dentitions was obtained by requiring the subjects to perform additional tests with greater numbers of mastications, until all had produced foods having very nearly a pulverization which would permit 78 per cent of the peanuts to pass through the 10 mesh screen. The exact number required was estimated by interpolation and was compared with the number of strokes required by the "standard" patient for achieving the same results. Thus, if 40 mastications were required by one subject for causing 78 per cent of the peanuts to pass through the 10 mesh screen, this figure would be compared with the 20 mastications needed by the "standard" patient to achieve the same pulverization.[4] The unknown subject would have a masticatory efficiency of 50 per cent.

IMPROVEMENTS OF WAX IMPRESSION COMPOSITION

The previous composition of wax had to be warmed to 40° C. before usage.[1] Flaming the wax failed to give duplicable results with different operators. Immersion in water sometimes caused separation of the wax, and ovenheating was inconvenient. The wax was made softer by raising the percentage of low melting components and was tested on 50 dental students. Their masticatory area was correlated with masticatory performance, but the coefficient was 0.6 rather than the 0.8 which had been obtained previously.[3] Changes were needed in the Cellophane insert because the registration often tore during tests on some patients. A study of different thicknesses of polyethylene film, polyvinyl plasticized film, and the original Cellophane film indicated that a thicker polyethylene film gave better correlation between performance and area measurements and eliminated wastage due to rupture of the film. In the same investigation the hardness of the wax was adjusted and its optimum opacity was selected. In the procedure finally selected two to three kilograms of wax are prepared from the following percentage mixture of components:

White microcrystalline wax m.p. 145 to 50°, No. 7819*------	42.8 per cent
Black wax, No. 9540* ----------	4.8
Black acara wax, No. 9840* ----------	23.8
Paraffin, Amber, 50-52° C.**----------	28.6
	100.0 per cent

The wax was melted over a flame in a metal vessel, stirred well and allowed to cool to a temperature of 75° C. A drinking glass filled with water and ice was dipped twice into the molten wax, removed, and allowed to stand until the wax was hard. Sheets of wax were cut from the sides of the glass and residual

*Obtained from H. B. Morse Co., Newton Lower Falls, Massachusetts.
**Obtained from Howe & Trench, Boston, Mass.

moisture allowed to evaporate. A sheet of 0.010 inch clear polyethylene film* was sandwiched between two sheets of wax. Two by one inch strips were cut from this material and checked for opacity on the area meter. High or low opacities were rejected. Satisfactory registrations were placed between aluminum foil sheets and kept in a cool place without refrigeration.

The area of each side of the dentition posterior to the cuspid was registered in duplicate on the wax. The area meter was set to zero on the blank wax and adjusted thereafter to read in square millimeters.[3] It was found that the area reading increased over a period of 24 hours if the strip was kept in a warm place and remained the same if the strip was kept chilled. This observation was interpreted to indicate relief of strain in the wax. Prediction Table II in this paper is based either on readings obtained within 1 hour or 24 hours after the registrations have been taken.

The total molar plus premolar mesiodistal lengths were measured with a millimeter scale. The mesiodistal length of any gaps in the food platform area were recorded, and the mesiodistal length of molar imprints was noted. From observations on the wax, the number and type of teeth in occlusion on upper and lower jaws were recorded and converted to tooth units per half dentition, assigning a score of one to each molar pair and one-half to each premolar pair.

RESULTS

The extent of correlation between different variables was estimated by means of the coefficient mean square contingency, c, after correction for the number of categories.[6] Values for this correlation are presented in Table I.

TABLE I

CORRELATION BETWEEN MASTICATORY FUNCTION MEASUREMENTS

	$P_{P\text{-}10}$	$P_{C\text{-}5}$	E_P	A	MIL	TU
$P_{P\text{-}10}$	.92					
$P_{C\text{-}5}$	.89	.84				
E_P	.84	.73	--			
A	.82	.75	.85	.88		
MIL	.80	.74	.68	.53	--	
TU	.76	.71	.22	.56	.84	--

The self-correlation coefficient between performance tests with peanuts (0.92) is higher than that for carrots (0.84) which indicates that the peanut test is more duplicable, presumably because of the greater uniformity in size and texture which peanuts obviously possess. The findings for masticatory performance with carrots were compared with those with peanuts. The carrot data are based on percentage of food passing the 5 mesh screen after 40 mastications, whereas the peanut performance is based on that passing the 10 mesh screen after 20 mastications; hence similarity of the percentages do not indicate that both foods have the same resistance to crushing. The relationship was

*Obtained from Plax Corp., 76 Tolcott Road, West Hartford, Conn.

apparently linear, and the correlation coefficient (Pearson r) between these two variables was found to be 0.84. This finding confirms a previous finding of a high correlation (0.79) between peanuts and another tough food, coconut.[4] Both procedures apparently measure essentially the same phase of mastication, and the more duplicable test, P_P, was selected as the criterion of chewing ability.

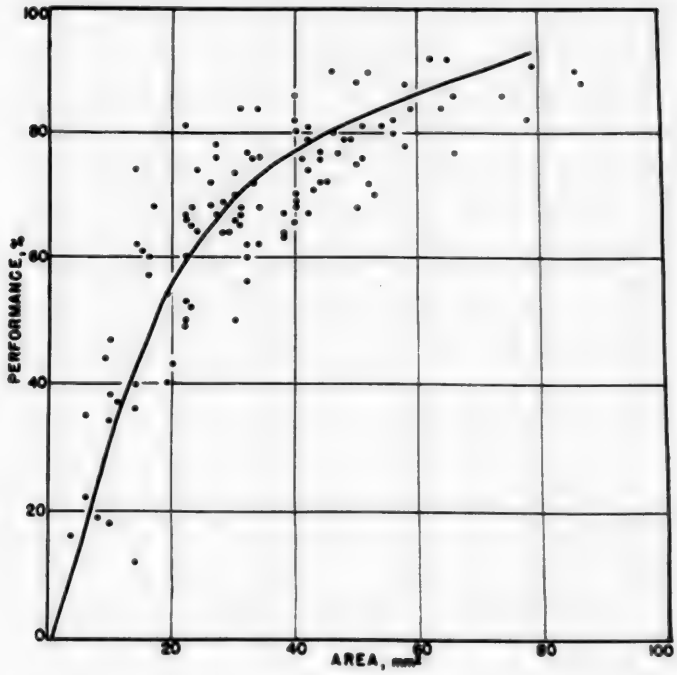


Fig. 1.—Relationship between masticatory performance and food platform area. The variables are correlated in a nonlinear manner.

Masticatory performance (P_P) was related to food platform area in a curvilinear manner (Fig. 1). Conventional means were tried first for discovering the mathematical relationship between these two variables. The relationship was nearly linear on a logarithmic-arithmetic chart, indicating fit for an exponential equation. This equation would predict values for performance higher than one hundred or less than zero, which was absurd. On a logarithmic-logarithmic plot the data was nearly linear, but the equation derived therefrom would suffer from the same absurdity. The use of the probability scale for performance served to eliminate the problem of performance values above one hundred or less than zero, but the relationship was still nonlinear. It was only when the logarithm of the area was plotted against the probability of performance that the relationship became linear, and the equation gave reasonable solutions at high and low area values (Fig. 2). Another reason for selecting the probability function of performance was its validity in relating performance to the number of chews in a previous investigation.[4]

PREDICTION OF MASTICATORY EFFICIENCY, E_P

The food platform area (A), mesiodistal molar imprint length (MIL) and tooth units were reasonably well correlated with the masticatory performance of peanuts. Attempts were made to develop an equation directly for predicting masticatory performance from these three variables, but the calculations were unwieldy because of the logarithmic-probability correlation between performance and the other three variables. It seemed to be desirable to select a parameter which would be related to area, molar imprint length and tooth units in a less cumbersome manner. Perhaps masticatory efficiency (E_P) would be suitable.

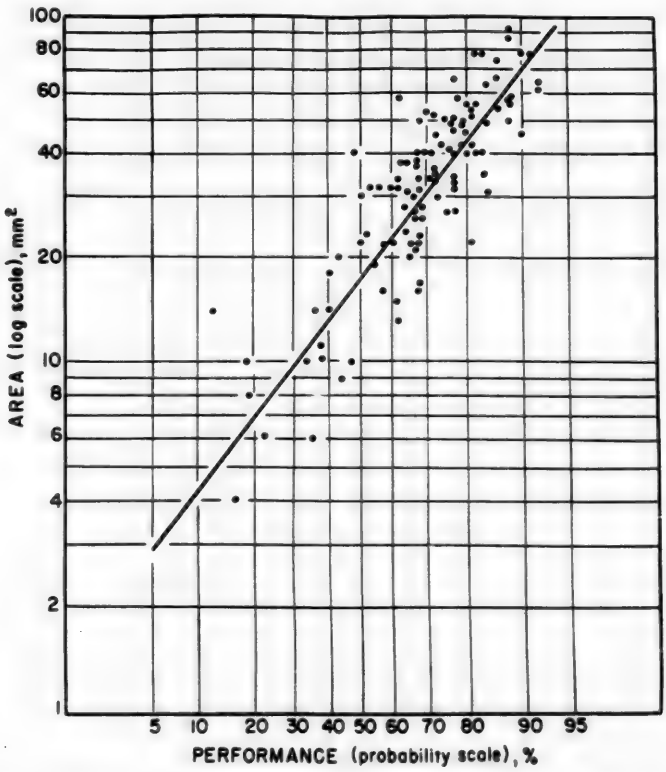


Fig. 2.—Masticatory performance vs. food platform area (probability-logarithmic scales). The probability function of performance is linearly related to the logarithm of food platform area.

Both it and area appeared to be related to performance by a logarithm-probability relationship, and hence efficiency and area should be related to each other in a linear manner. Fifty-four tests on masticatory efficiency were made in the manner already described. These half-dentitions were selected in order to obtain distribution of values in the high and low range, and to avoid the large numbers of cases in the middle range, which were obtained with the random selection of cases.

It can be seen from Table I that area is well correlated with efficiency. Molar imprint length is less well correlated, and tooth units possess a very low correlation. The relation between area and efficiency is linear (Fig. 3). Efficiency was also related linearly to molar imprint length. Since efficiency is correlated with area and with molar imprint length more highly than these two variables are related to each other, it can be assumed that a regression equation involving both will provide a better prediction of efficiency, than will either factors alone. Beta coefficients were calculated[7] and the following equation was developed from 24 hour area readings:

$$E_P = 1.89A + 0.95\ MIL - 3$$

E_P was calculated from this equation and compared with the measured efficiency. The correlation coefficient between the two sets of data was 0.90, which is approximately the maximum which could be expected.

The formula for E_P permitted us to solve the mathematical relationship between P, A and MIL graphically. E_P was related to P in the following manner. The probability function of $P = \frac{\log E - 1.583}{0.542}$. Substitution for E_P from equation (1) gives $\Phi P = \frac{\log (1.89A + 0.95\ MIL - 3) - 1.583}{0.542}$.

Table II is a prediction table for efficiency and performance which gives solutions for equation 2 and 4 based on values for area (either at 1 or 24 hours) and molar imprint length. It is employed by taking readings of A and MIL on the preferred side of the dentition of a subject. As an example consider an impression with A as 33 mm.^{2} and MIL, 20 mm. Read down the 33 mm. column and across the 20 mm. column to their intersection in order to obtain the figure for predicted efficiency.

DISCUSSION

The most important determinant of masticatory efficiency appears to be food platform area. It is best correlated with efficiency and when molar length is held constant it is still related to efficiency. For example, there were about 70 half dentitions with MIL, 20 to 22 mm. Among this group the area ranged from 13 to over 90 sq. mm. and was still related somewhat to performance. Yet when 24 cases having 35 to 40 mm.^{2} of area were selected, MIL was found to range from 7 to 33 mm. and was no longer related to performance.

This might lead to the conclusion that MIL is not needed in the prediction equation. Its value appears as partial correction of certain inadequacies in the area test. Food platform area measurements are excessively affected by the shape of molar cusps. Newly erupted teeth usually have area readings which are too low for the performance that is observed. Teeth with attrition have erroneously high areas, which is demonstrated in Fig. 2 in which areas over 50 sq. mm. do not show the expected higher masticatory efficiency. It is best to adopt the rule of assigning 50 sq. mm. as the maximum value for use in predicting efficiency.

The criterion formerly employed by the Armed Services for chewing ability was based on the number of tooth units present. Three and one-half masticating tooth units per mouth corresponding to 3 molar and 1 premolar pairs in occlusion were specified as being required for adequate chewing ability. This specification proves to be unsuitable because of failure to set forth the location of teeth in the mouth. If all pairs of teeth were on the same side of the mouth, the efficiency for the mouth would average over 100 per cent. If the occluding pairs were divided about equally on either side, the mouth would be likely to

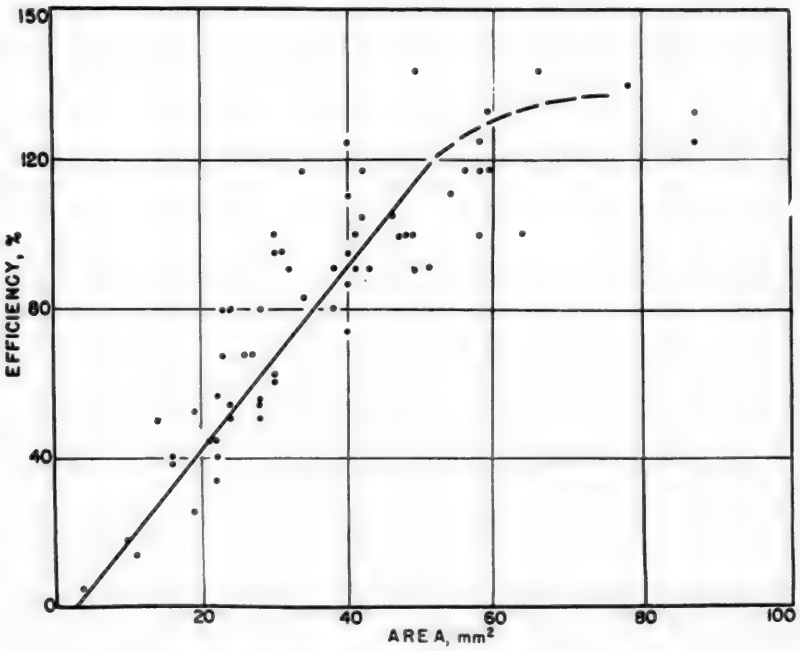


Fig. 3.—Masticatory efficiency is linearly related to food platform area.

have about 50 per cent efficiency. When the position of molar and premolar pairs is prescribed, the index is still too crude for a good screening device. The lower border of normal performance has been tentatively set at 69 per cent from findings on 100 half-dentitions with two molar and two premolar pairs.[8] Half of the 200 half-dentitions in this study had 3 tooth units present, yet performance among this half ranged from 38 to 93 per cent. If 3 tooth units were required on the best half dentition, this screening would include 94 per cent of persons with "standard" performance and 56 per cent of those with "substandard" chewing ability. The screening is imperfect and is not improved by raising the requirements to 3.5 units, for with this specification only one-fifth of those with standard chewing ability or better are included. The unsuitability of tooth units is further disclosed from its low correlation with efficiency (0.22).

TABLE II

PREDICTION OF MASTICATORY PERFORMANCE AND EFFICIENCY FROM FOOD PLATFORM AREA AND MESIODISTAL IMPRINT LENGTH

AREA		PREDICTED EFFICIENCY AND PERFORMANCE MOLAR IMPRINT LENGTH								
IMMEDIATE READING	24 HOUR READING	0	5	10	15	20	25	30	35	40
	0	*0*	*2*	*6*	*11*	*16*	*21*	*26*	*30*	*35*
		0	1	7	16	25	32	38	43	47
	3	*6*	*7*	*12*	*17*	*22*	*26*	*31*	*36*	*41*
		7	9	18	26	33	38	43	48	52
1	6	*11*	*13*	*18*	*22*	*27*	*32*	*37*	*42*	*46*
		16	21	28	33	39	44	49	53	56
4	9	*17*	*19*	*24*	*28*	*33*	*38*	*42*	*47*	*52*
		26	29	36	40	45	50	53	56	59
7	12	*23*	*24*	*29*	*34*	*39*	*44*	*48*	*53*	*58*
		34	36	41	46	50	54	57	60	63
10	15	*28*	*30*	*35*	*40*	*44*	*49*	*54*	*59*	*63*
		40	43	47	51	54	58	61	64	66
13	18	*34*	*36*	*40*	*45*	*50*	*55*	*60*	*64*	*69*
		46	48	51	55	58	61	64	66	68
16	21	*40*	*41*	*46*	*51*	*56*	*60*	*65*	*70*	*75*
		51	52	56	59	62	64	66	68	70
19	24	*45*	*47*	*52*	*57*	*61*	*66*	*71*	*76*	*80*
		55	56	59	62	64	67	69	71	72
22	27	*51*	*53*	*58*	*62*	*67*	*72*	*76*	*81*	*86*
		59	60	63	65	67	69	71	73	74
24	30	*57*	*58*	*63*	*68*	*73*	*78*	*82*	*87*	*92*
		62	63	66	68	70	71	73	74	76
27	33	*62*	*64*	*69*	*74*	*78*	*83*	*88*	*93*	*97*
		65	66	68	70	71	73	75	76	77
30	36	*68*	*70*	*74*	*79*	*84*	*89*	*94*	*98*	*103*
		68	68	70	72	74	75	76	77	78
33	39	*74*	*75*	*80*	*85*	*90*	*94*	*99*	*104*	*109*
		70	70	72	74	75	76	78	79	80
36	42	*79*	*81*	*86*	*91*	*95*	*100*	*105*	*110*	*114*
		72	73	74	75	77	78	79	80	81
39	45	*85*	*87*	*92*	*96*	*101*	*106*	*112*	*115*	*120*
		74	74	76	77	78	79	80	81	82
42	48	*91*	*92*	*97*	*102*	*107*	*112*	*116*	*121*	*126*
		75	76	77	78	79	80	81	82	83
44	50 above	*94*	*96*	*101*	*106*	*110*	*115*	*120*	*125*	*130*
		76	77	78	79	80	81	82	83	84
47		*98*	*99*	*104*	*109*	*114*	*118*	*123*	*128*	*132*
		77	78	79	80	81	82	83	83	84
50 above		*103*	*105*	*110*	*115*	*119*	*124*	*129*	*134*	*138*
		78	79	80	81	82	83	83	84	85

Values for efficiency are in italics.
Values for performance are in roman.

There were several dentitions having diastemas because of extraction. It seemed logical to expect that missing teeth in the first molar region would impair mastication beyond that predicted from the change in A and MIL. The division in food table might give difficulties in food manipulation. No evidence could be found for this assumption. Twenty-four half-dentitions had diastemas ranging from 2 to 18 mm. in the first molar region. Prediction of chewing ability was poor for this group, but it was not consistently high.

The ability to predict masticatory efficiency with reasonable precision from area and molar imprint length measurements indicates that the important factors are known for mastication by this group of young adults. Other factors such as maximum force, saliva flow, mandibular motion, balance of occlusion may become important among groups of persons who differ in age, who have a deciduous or artificial dentition or are affected by periodontal disease or other pathological conditions. Apparently among young adults these factors do not have much influence on their ability to masticate food.

SUMMARY

The masticatory performance of 103 persons was determined on right and left halves of their dentitions with both peanuts and carrots as test foods. Masticatory efficiency was obtained for 54 half-dentitions. These function measurements were compared with food platform area, tooth units in occlusion, and mesio-distal length of molar imprints for the same half dentitions.

Masticatory performance was correlated with all three variables but in a nonlinear manner. Masticatory efficiency was well correlated with food platform area, in a linear manner, less well related to molar imprint length and poorly related to tooth units. A prediction equation was worked out for performance and efficiency from area and molar imprint length. This predicted efficiency agreed with the actual efficiency with a correlation coefficient of 0.90.

Apparently the food platform area and the size of molars are the most important determinants for chewing ability among adults with a normal or defective natural dentition.

REFERENCES

1. Schutz, F.: Dentition and Digestion, *Z. f. Hyg. und Infectionskrankheiten* **95**: 279, 1922.
2. Dahlberg, B.: The Masticatory Effect, Lund Hakan Ohlssons Boktryckeri, 1942.
3. Yurkstas, A., and Manly, R. S.: Measurement of Occlusal Contact Area Effective in Mastication, *Am. J. Orthodontics* **35**: 185, 1949.
4. Manly, R. S., and Braley, L. C.: Masticatory Performance and Efficiency, *J. D. Res.* **29**: 448, 1950.
5. Yurkstas, A., and Manly, R. S.: Value of Different Test Foods in Estimating Masticatory Ability, *J. Applied Physiol.* **3**: 45, 1950.
6. Peters, C. C., and Van Voorhis, W. R.: Statistical Procedures and Their Mathematical Basis, New York, 1940, McGraw-Hill Book Co., pp. 391-399.
7. Arkin, H., and Colton, R. R.: An Outline of Statistical Methods, Ed. 4, 1939, Barnes and Noble, Inc., p. 96.
8. Manly, R. S.: Practical Applications of Research on Mastication, Monthly Research Reports of the Office of Naval Research, Department of the Navy, Feb. 1, 1950, p. 16.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
PROGRAM OF THE CHICAGO SECTION FOR THE YEAR 1950-1951

COMPILED BY FRANK ORLAND, EDITOR, CHICAGO SECTION

AT THE five meetings of the Chicago Section held during the year 1950 to 1951, the following twenty papers were presented:

October 27, 1950.—Adrenal Cortical Physiology and Its Relationship to Oral Structures. Irving B. Fritz, University of Chicago, Zoller Dental Clinic.

Studies on the Number of Strongly Acidogenic Bacteria and Their Relation to Clinical Caries. Dorothy R. Rizzo and Evelyn B. Tilden, Northwestern University Dental School.

Growth of the Upper Face. Harry Sicher, Loyola University School of Dentistry.

November 24, 1950.—Investigations with Radio-Active Anti-Caries Agents. William W. Wainwright, College of Dentistry, University of Illinois.

A Case of Acquired Cleft Palate and Its Prosthetic Repair. Robert M. Appleman, University of Chicago, Zoller Dental Clinic.

Aureomycin: Its Use in Treatment of Acute Infectious Stomatitis in Children. Cosmo Castaldi and Gordon H. Rovelstad, Northwestern University Dental School.

Unusual Findings in Gingival Biopsy. Balint Orban, Colorado Dental Foundation, Colorado Springs, Colo.

January 26, 1951.—The Bisulfite Binding Index as Related to Caries. Ben F. Gurney and Gustav W. Rapp, Loyola University School of Dentistry.

Collagenase-Like Activity in a Salivary Fraction. Leo M. Sreebny and Milton B. Engel, College of Dentistry, University of Illinois.

Traumatic Bone Cysts: Clinical Pathology. Eli Olech, College of Dentistry, University of Illinois.

Traumatic Bone Cysts: Pathogenesis. Harry Sicher, Loyola University Dental School; and Joseph P. Weinmann, College of Dentistry, University of Illinois.

A Three-Minute Résumé of Thirty Years of Chicago I. A. D. R. History. Frank J. Orland, University of Chicago, Zoller Dental Clinic.

April 27, 1951.—Electron Microscopic Studies of Connective Tissue. Friedrich Wassermann, Argonne National Laboratory and University of Chicago, Zoller Dental Clinic.

The Validity of Alizarin Red S as a Vital Stain in Deposition of Bone as Compared With Radio Calcium (Ca^{45}). Joseph R. Jarabak, Northwestern University Dental School.

Roentgenographic Studies of the Jaws in Sickle Cell Anemia. Irwin B. Robinson and Bernard G. Sarnat, College of Dentistry, University of Illinois.

Growth Changes in the Otic Region. Harry Sicher, Loyola University School of Dentistry.

May 24, 1951.—Research Activities of the Bureau of Chemistry of the A.D.A. J. Roy Doty, Bureau of Chemistry, American Dental Association.

A Study of Sutural Facial Growth by Means of Metallic Implants. Benjamin J. Gans and Bernard G. Sarnat, College of Dentistry, University of Illinois.

Effect of the Surgical Removal of the Condyle on the Growing Mandible: An Experimental Study in the Macaca Rhesus Monkey. Bernard G. Sarnat and Milton B. Engel, College of Dentistry, University of Illinois.

Progress Report on Mass Professional Education Including Research. Saul Levy, Telephone Extension Program, College of Dentistry, University of Illinois.

NOTICE TO SUBSCRIBERS

Librarians of teaching institutions and research organizations in several countries of Europe and the Orient are requesting back issues of the JOURNAL OF DENTAL RESEARCH.

During the war many countries were unable to receive scientific literature. The desperate need for scientific periodicals to aid intellectual construction in the war-torn countries has been vividly apparent by the numerous requests from these folks.

While we anticipated these requests to some extent and built up a reserve of the volumes published during the war, the requests which concerned us most were those received from libraries which are unable to finance the reconstruction of their vast number of volumes.

The William J. Gies Foundation for the Advancement of Dentistry has placed at our disposal a sum to aid the above-mentioned institutions. The sum will pay transportation from the subscriber and institution wishing to donate back issues for this cause.

It will be greatly appreciated if you will take inventory of your personal or institutional library and send any copies of the JOURNAL OF DENTAL RESEARCH which can be spared. They will be filed in proper sequence on our shelves. They will be sent without charge to those libraries, who in our belief are deserving but financially unable to pay and are urgently in need of this scientific material.

Send your contribution, or write for shipping instructions to—

J. Frank Hall, D.D.S.
Business Manager
JOURNAL OF DENTAL RESEARCH
538 North Berwick Avenue
Indianapolis 22, Indiana

INDEX

Volume 30, 1951

ABRASION: tooth surface; nylon and natural bristles, 522*
Acid: lactic, 100
——: production; different foods, 495*
——: ——; vitamins, 470*
——: ——; carbohydrate- and acid-containing substances, 126
——: ——; saliva; effects of metallic salts, 494*
——: retention; gold castings, 599*
——: pyruvic, 100
Acrylic: denture bases; fit, 496*
——: self-curing; pulp reaction, 498,* 524
Actinomyces: dentobacterial plaque, 88
Actinomycetes: carious lesions, 517*
ADAMS, E. C., JR.: caries; glucose degradation, 229
ADDELSTON, H. K.: x-ray diffraction; microcamera; tooth structure, 439, 476*, 478*
ADLER, P.: fluorides; caries experience, Hungary, 368
AFONSKY, D. A.: caries; Central China, 53, 463*
Agar-agar gel: dimensional changes, 281
Age: vertical dimension, 504*
ALDERMAN, N. E.: glossopyrosis; vitamin B_{12}, 515*
Aleut: dentition, 815
Alizarin: bone growth, 512*
——: bone mineralization, 511*
Alkaline phosphatase: gingiva, 461*, 792
Alloy particles: amalgam, 499*, 845
Alveolo-dental joint: examination technique, 638
Alveolar process: regeneration, 604
Amalgam: effect on dentin, 514*
——: physical properties, 499*, 845
Amblystoma punctatum: mandible development, 490*, 575
AMLER, M. H.: dentin penetration; effect of chemicals, 304*
Ammonia: anaerobic glycolysis, 656
——: lactobacillus, 470*, 542
——: saliva, 385
Ammoniated dentifrice: acid production; dental plaques, 495*
——: caries, 645
——: caries; hamster, 393
——: clinical test, 496*
——: lactobacillus counts, 503*
——: periodontal disease, 516*
——: urea and ammonium salt content, 388
Ammonium compounds: quaternary; dentifrices, 558
——salts: ammoniated dentifrices, 388
Amylase; saliva; sodium fluoride activation, 599*
Anemia: sickle cell; jaw studies, 520*
Anesthetic: local; intracaine amide, 462*
——: ——; levo-arterenol; vasoconstriction, 462*, 870
——: ——; physiological effects, 510*
——: ——; preliminary study, 463*
Anodic decalcification: teeth, 303*
Antibiotic levels: saliva, 495*
——: bactericidal effect, 481*
——: lactobacillus counts, 470*
——: fusobacteria; sensitivity, 799
——; see also specific names
ARMSTRONG, W. D.: fluoride; growing skeleton, 467*
——: radiocalcium; marginal adsorption; filling materials, 473*
Arthritis: dental foci, 504*
Articulation change: processing dentures, 500*
Attrition: eruption and growth rate; rat incisor, 521*
Aureomycin: herpetic gingivo-stomatitis, 459*
AVERY, J. A.: embryology; teeth, 490*
AVERY, J. K: hamster; caries; pouchectomy, 509*

BACTERIA: acid production, 100
——: antibiotic effects, 481*
——: calcium carbonate crystals, 642
——: dentinal caries, 766
——: mucinous polysaccharide production, 303*, 522*
——: see also specific names
BAKER, C. F.: silicate cements; physical properties, 145
BARBER, T. K.: dentin; amalgam effects, 514*
BARICH, F. T.: Class I malocclusion; comparative studies, 505*
BARTELS, H A.: calcium carbonate crystals; bacteria, 642
BARTELSTONE, H. J.: enamel; tooth penetration; radioactive iodine, 304*, 480*, 728
BAUME, L. J.: jaw growth; tooth development and eruption, 502*
——: postnatal growth; mandible; Macaca mulatta, 502*
——: ——; maxilla; Macaca mulatta, 501*
BECKS, H.: topical fluoride application; young adults, 757
BELGOROD, H. H.: dentin permeability; radioactive materials, 480*
BELL, M. E.: cetylamine; toxicity, 214
Bending properties: dental gold wires, 290
BERGGREN, H.: enamel permeability; fluorine, 169
——: ——; sugar solutions, 161
BERZINSKAS, V. G.: caries; ionizing radiation, 787
——: ——; rat; bilateral symmetry, 62

*Abstract only.

BESIC, F. C.: caries; in vitro, 483*, 708
BIBBY, B. G.: acid production; foods, 495*
——: ammoniated dentifrices; lactobacillus counts, 503*
——: caries production; hamsters; decalcification potentials, 485*
——: chlorophyll; effect on oral organisms, 469*
——: Presidential Address, I.A.D.R., 455
Bicarbonate; salivary buffer effect, 403
BLAYNEY, J. R.: fluoridation; caries experience; 12-, 13-, 14-year-olds, 465*, 670
BLECHMAN, H.: aqueous urea solution; endodontics, 503*
Blood: fluoride, 182
BOMAN, V. R.: mandibular condyle position; orthodontic treatment, 507*
Bone: fluoride, 467*
——: growth, 487*
——: ——; alizarin, 512*
——: radiocalcium uptake, 511*
BOWLER, F. T.: dental survey; children, 303*
BOYD, D. J.: dental caries; progression; second permanent molars, 465*
BRAUER, G. M.: self-curing resins chemistry, 497*
Bridgework: functional evaluation, 475*
BRIGHT, P. J.: vertical dimension; age, 504*
BRUCKNER, R. J.: caries prevalence; school children, 464*
BRUDEVOLD, F: ammoniated dentifrice; acid production; dental plaques, 495*
——: enamel surface; chemical studies, 477*
——: micro antimony electrode; intraoral pH, 523*
BRUMFIELD, R. C.: testing; dental gold alloys, 499*
Brushing: ammoniated dentifrices; caries, 393, 486*
——: see also dentifrice
Bur: cutting efficiency, 497*, 854
——: rotational speed; temperature rise, 497*, 737
BURNETT, G. W.: bacteriological studies; advancing dentinal lesions, 766
——: chemical compounds; dentin effects, 477*
BUSH, S. H.: gold wires; section size; mechanical properties, 745
——: wrought gold wires; section size, 500*
BUTCHER, E. O.: denervation and ischemia; dental effects, 265

CABRINI, R. L.: alkaline phosphates; gingiva, 28
Calcium: blood; vitamin D and parathyroid effects, 487
——: carbonate crystals: bacteria, 642
——: histochemical reaction, 489*
——: radioactive, 304*, 473*, 511*
CALLANDRA, J. C.: caries; glucose degradation, 229
CANFIELD, R. C.: radiation injury; teeth, 477*
CANTRIL, S. T.: radiation injury; teeth, 477*
Carbohydrate: enamel, 480*
Carbohydrate metabolism: saliva, 494*
Caries: acid production, 100
——: actinomyces, 88, 517*
——: albino rat; incidence, 382
——: ammoniated dentifrice, 388, 393, 495*, 503*, 516*, 645
——: artificial, 483*, 708
——: Central China, 53, 463*
——: children; Willamette Valley, 509*
——: chlorophyll, 510*
——: complex fluorides, 466*, 524
——: decalcification potential; foods, 485*
——: dentin; bacteriological studies, 766
——: dentobacterial plaque, 399, 423, 482*
——: diet, 53, 778
——: enamel matrix, 690
——: evaluation; reproducible method, 346
——; fluoride, 225, 514*
——: ——; complex, 466*
——: fluoridation; Evanston, 465*, 670
——: ——; Greece, 225
——: glucose degradation, 229
——: Greece; fluorine, 225
——: hamster, 139, 393, 484,* 485,* 509,* 778
——: histology, 690
——: incidence, 62, 464,* 465,* 512*
——: in vitro, 482,* 483,* 708
——: iodoacetic acid, 203
——: ionizing radiation, 787
——: nitrogen phosphorus, 97
——: physicochemical factors, 235
——: progression; second permanent molars, 465*
——: psychiatric patients, 806
——: quaternary ammonium compounds, 558
——: rat; bilateral symmetry, 62
——: ——; buccal and lingual surfaces, 484*
——: salivary gland extirpation, 486*
——: selenium, 523*
——: streptococcus salivarius, 116
——: sympathicotony; salivary glands, 783
——: see also decalcification
Carious areas: photoelectric determination, 513*
CARRANZA, F. A.: alkaline phosphatase; gingiva, 28
Carcinoma: oral; exfoliative cytology, 308
Carotid sinus: effects on Ludwig's angina, 489*
CARTER, W. J.: dentin; bound cholesterol, 837
——: dentinal protein; preservatives, 840
CASTALDI, C. R.: infectious gingivo-stomatitis; aureomycin, 459*
CATTONI, M.: lymphocytes; healthy gingiva, 486,* 627
Cavity preparation: enamel walls, 473*
Cephalometric radiographic studies; facial anomalies, 506*
Cetylamine: enamel decalcification, 218
——: toxicity, 214
CHAWLA, T. N.: cortisone acetate; periodontal tissues, 461*

Chelating agents: organic; demineralization, 472*
CHEN, E.: caries production; hamsters; decalcification potential, 485*
CHERNAUSEK, D. S.: ammoniated dentifrices; hamster caries, 393, 486*
——: ——; periodontal disease, 516*
——: hamster caries; dietary sugar levels, 778
——: periodontal disease; hamster, 802
Chewing ability: denture wearers, 314
Children: caries incidence, 464,* 509,* 512*
——: dental survey, 303*
——: salivary yeasts, 468*
CHILTON, N. W.: dolorimetric evaluation; dental patients, 822
China: caries, 53, 463*
Chlorophyll: caries inhibition, 510*
——: lactobacilli, 465*
——: oral organisms, 469*
Cholesterol: dentin, 837
CHRISTIAN, K.: bactericidal effect; antibiotics, 481*
CLARK, W. D.: caries: ionizing radiation, 787
Cleft palate management: 507*
CLEGG, C. T.: saliva; ammonia production, 385
CLUBB, R W.: hamster; caries; pouchectomy, 509*
COBE, H. M.: quaternary ammonium compounds; dentifrices, 558
COHEN, M.: antibacterial root canal cement, 475*
——: aqueous urea solution; endodontics, 503*
Collagenase: activity; saliva, 493*
——: oral environment, 493*
Condyle: removal; effect on mandible growth, 520*
——: resection; upper face growth, 491*
Connective tissue ground substance; gingivae, 792
——: tooth eruption, 322
COOLIDGE, T. B.: caries; nitrogen and phosphorus content, 97
——: enamel; carbohydrate content, 480*
COOPER, E. N.: acrylic denture bases; fit, 496*
Cornification: carcinoma, 308
——: leukoplakia, 260
——: oral mucosa, 4, 12
Cortisone: effect on rat fetuses, 488*
——: effect on young rats, 488*
——: periodontal tissues, 461*
COSTICH, E. R.: gingival disease; young women, 461*
COWIE, D. B.: fluorine adsorption; specific surface area, 467*
CROWELL, W. S.: amalgams; variations in physical properties, 499*, 845
CROWELL, G. E.: viral agent; dentinal destruction, 82
——: photoelectric determination; carious lesions; areas, 513*
CURBY, W. A.: radiopacity; intraoral method, 473*
Cysts: traumatic, 517*

DALTON, P. J.: dental disease; psychiatric patients, 806
DAM, H.: enamel depigmentation; protective effect of methylene blue, 508*
DAVIES, G. N.: ammonium ion dentifrice; caries control, 645
DEAKER, E. M.: cetylamine; toxicity, 214
Decalcification: anodic, 303*
——: enamel; fluorides; zinc-ferro-nitrate, 75, 189, 195
——: polycarboxylic acids, 484*
——: polyphosphates, 484*
——: versene, 484*
Defense mechanisms: mouth, 494*
Delinquency: tooth fracture, 513*
DE MENT, J.: anodic decalcification; teeth, 303*
——: natural radioactivity; teeth, 302*
Demineralization: organic chelating agents, 472*
Denervation: dental effects, 265
Density: rodent enamel, 828
Dentifrice: ammoniated, 388, 393, 486*, 495*, 496*, 503*, 516*, 645
——: quaternary ammonium compounds, 558
Dentobacterial plaque: acid production; ammoniated dentifrices, 495*
——: actinomyces, 88
——: micrococcus lactilyticus, 423, 482*
——: tetradecylamine effect, 399
Dentin: amalgam effect, 514*
——: cholesterol; bound, 837
——: destruction; viral agent, 82
——: effects of chemical compounds, 477*
——: fluoride content, 172, 360
——: lipids, 599*
——: permeability; radioactive materials, 480*
——: protein; preservatives, 840
——: ——; recovery, 565
Dentition: Aleut, 815
Denture: processing; change of articulation, 500*
——: bases: plastic teeth, bonding, 501*
——: wearers: chewing ability, 314
DETWILER, S.: mandible development, Amblystoma punctatum, 490*, 575
DEXTER, H.: viral agent; dentinal destruction, 82
Diet: caries, 53, 778
DIRKS, O. B.: caries evaluation; reproducible method, 346
DOBBS, E. C.: new local anesthetic agent, 463*
Dolorimetric evaluations; dental patients, 822
DOMM, L. V.: cortisone; rat growth, 488*
——: ——; —— fetuses, 488*
DONNELLY, D. F.: mucinous polysaccharide; oral bacteria, 522*
DOWNS, W. L.: complex fluorides; caries inhibition, 466*
DRIAK, F.: dental foci and arthritis, 504*
DUNNING, J. M.: dental disease, psychiatric patients, 806

EAR disturbance: masticatory apparatus, 523*
Edentulous jaws: x-ray studies, 504*
Editor's Viewpoint: 1, 157, 305, 453, 601, 753
EDWARDS, R. W.: edentulous jaws; x-ray study, 504*
Efficiency: masticatory performance, 874
EICKENBERG, C. F.: pleuropneumonia-like organisms; saliva, 415
Electron microscopy: enamel, 478*
——: thin sections, 476*
ELLINGER, F.: dental caries ionizing radiation, 787
ELLIOTT, H. W.: Ludwig's angina; carotid sinus studies, 489*
ELLIS, R. G.: sodium fluoride; dental calcium fluoride content, 360
Embryology: teeth, 490*
Enamel: caries; nitrogen and phosphorus, 97
——: carious; carbohydrate content, 480*
——: cuticle; microstructure, 478*
——: ——; fluorides; zinc-ferro-nitrate, 75, 189, 195
——: decalcification; fluorine, 177, 363, 515*
——: ——; surface active agents, 218
——: depigmentation; vitamin E; methylene blue protection, 508*
——: density distribution; x-ray diffraction; rodent, 828
——: fluorides, 169, 172, 177, 360, 466*, 467*, 515*
——: matrix, 478*
——: ——; histologic technique, 431
——: organic framework; electron microscopy, 478*
——: permeability, 161, 169, 480*, 728
——: proteinaceous materials, 479*
——: rodent; physical properties, 445
——: solubility; sodium monofluorophosphate, 466*
——: surface; chemical studies, 477*
——: x-ray diffraction, 478*
——: walls; cavity preparation method, 473*
Endodontics: urea solution, 503*
——: see also root canal
ENGEL, M. B.: collagenase activity; saliva, 493*
——: connective tissue ground substance; changes; tooth eruption, 322
——: mandible growth; condyle removal, 520*
——: parathyroid extract; serum mucoprotein, 492*
ENGLISH, J. A.: ionizing radiation; oral effects, 33
——: selenium; caries incidence, 523*
ENNEVER, J.: actinomyces; dentobacterial plaque, 88
——: dentobacterial plaque; micrococcus lactilyticus, 423, 482*
Enzyme inhibitors: lactobacillus counts, 470*
EPSTEIN, S.: levo-arterenol; vasoconstrictor activity, 462*, 870
Eruption: teeth; connective tissue ground substance changes, 322
Exfoliative cytology: leukoplakia, 260
——: oral carcinoma, 308
——: —— mucosa, 4, 12

FACIAL structures: occlusal planes; analysis, 505*
FANKUCHEN, I.: x-ray diffraction; microcamera; tooth structure, 439, 476*, 478*
Filling materials: radioactive calcium; adsorption, 473*
FISCHER, C. J.: blood calcium and phosphorus; vitamin D and parathyroid effects, 487*
——: mineral stress; vitamin D; parathyroids, 508*
FISCHER, T. E.: bonding; plastic teeth; denture bases, 501*
FISHER, B. J.: lactobacilli; acid production; vitamins, 470*
FLEET, I. J.: Class I malocclusion; comparative studies, 505*
Fluoridation: caries experience; Evanston, Illinois, 465*, 670
——: three-year study; Ottawa, Kansas, 465*
Fluoride: acidulated; enamel decalcification, 363, 519, 757
——: adsorption; enamel, 467*
——: blood, 182
——: bone; growing, 467*
——: calcium fluoride content; teeth, 360
——: caries; topical; adolescence, 514*
——: caries experience, Greece, 225
——: —— —— Hungary, 368
——: complex, 466*, 524*
——: consumption; tooth content, 172
——: dentin effects, 477*
——: enamel decalcification, 75, 189, 195
——: enamel permeability, 169
——: —— solubility, 177, 515*
——: salivary gland, 516*
——: salivary gland changes, 712
——: sodium monofluorophosphate, 466*
——: teeth; content, 172
——: topical; lactobacillus counts, 251, 514*
——: topical application; young adults, 519*, 757
——: vegetable fluorine content, 466*, 676
Focal infection: diagnosis, 515*
——: experimental study, 481*
Foci: dental; diagnosis; therapy, 504*
Foods: decalcification potential; caries production, 485*
FORBES, J. C.: salivary acid production; effects of metallic salts, 494*
FOSDICK, L. S.: enamel permeability, 480*
——: —— solubility; fluorine, 177
——: lactobacillus counts; antibiotics; enzyme inhibitors, 470*
Fractured teeth: delinquency, 513*
FRANER, C. E.: caries; ionizing radiation, 787

FREEMAN, R. S.: analysis; facial structures; occlusal planes, 505*
FRIDLEY, H. H.: functional evaluation; bridgework, 475*
FRIEDMAN, B. K.: lipids; dental tissues, 599*
FRISBIE, H. E.: caries; enamel matrix, 690
——: caries lesions; production in vitro, 482*
Fusobacteria: antibiotics, 799

GAARD, R.: cultural characteristics; actinomycetes; lactobacilli; streptococci, 468*
GANS, B. J.: sutural facial growth; metallic implant studies, 506*
GARDNER, D. E.: fluoride; blood, 182
——: fluorides; caries complex; inhibition, 466*
——: sodium monofluorophosphate; salivary acid production; hydroxy apatite solubility, 466*
Gasserian ganglion: chromatolysis; pulp removal, 496*
Geographic variation: cariogenic effects, 485*
GEORGI, C. E.: Streptococcus salivarius; dental caries, 116
GERBER, W. E.: cephalometric radiographic studies; skeletal pattern, 506*
GERENDE, L. J.: caries; rat; bilateral symmetry, 62
GIBILISCO, J. A.: chromatolysis; gasserian ganglion; pulp removal, 486*
GILDA, J. E.: rodent enamel; physical properties, 445
——: ——; density distribution; x-ray diffraction, 828
GILLIS, R. E.: lactobacillus glycolytic enzymes, 471*
Gingiva: alkaline phosphatase, 28, 461*
——: glycogen, 792
——: histochemical evaluation, 460*, 792
——: histochemistry, 792
——: lactation, 19
——: lymphocytes, 486*, 627
——: nutritional deficiency, 19
——: pregnancy, 19
Gingival diseases: young women, 461*
—— fibers: role, 331
——: topography, 331
Gingivo-stomatitis: see herpetic gingivostomatitis
Glands: labial and buccal; origin and distribution, 587
GLASS, R. L.: yeasts; saliva; children, 468*
GLICKMAN, I.: cortisone acetate; periodontal tissues, 461*
——: histochemistry; gingivae, 460*, 792
Glossopyrosis: vitamin B_{12}, 515*
GLOWACZ, C. R.: mandibular condyle position; orthodontic treatment, 507*
Glucose degradation: caries, 229
Glycogen: gingivae, 792
Glycolysis: anaerobic; ammonia urea, 656
Glycolytic enzymes: lactobacillus, 471*
GOLDBERG, H. J. V.: acid production; foods, 495*
——: caries-producing hamsters; decalcification potential, 485*
Gold: alloys; dental; comparative testing, 499*
——: castings; acid retention, 599*
——: dental wires; tensile and bending properties, 290
GOLDMAN, H. M.: gingival fibers; topography and role, 331
Gold wires: section size; mechanical properties, 745
——: wrought; effect of section size, 500*
GORLIN, R. J.: vitamin B complex deficiency; mandibular joint and periodontal changes, 337
GRABER, T. M.: analysis; facial structures; occlusal planes, 505*
——: bilateral mandibular condylar resection; upper face growth, 491
——: cleft palate management, 507*
Gram reaction: mechanism, 471*
GRANADOS, H.: enamel depigmentation; protective effect of methylene blue, 508*
GREEN, R. W.: enamel decalcification; surface active agents, 218
GREENBERG, L. A.: salivary acidity; carbohydrate- and acid-containing substances, 126
GREEP, R. O.: mineral stress; vitamin D; parathyroids, 508*
——: blood calcium and phosphorus; vitamin D; parathyroid effects, 487*
GROHS, R.: maxillary orthopedics, prosthetics, 509*
GROSSMAN, L. I.: bactericidal effect; antibiotics, 481*
Ground substance: connective tissue, 322, 792
Growth: mandible; effect of condyle removal, 520*
——: mandible; Macaca mulatta, 502*
——: maxilla; Macaca mulatta, 501*
——: sutural; facial studies, 506*
——: upper face; condylar resection, 491*
GURNEY, B. F.: intermediary carbohydrate metabolism; saliva, 494*

HACKEDORN, H. M.: mucinous polysaccharides; streptococci, 302*
HADJIMARKOS, D. M.: dental caries; Willamette Valley, 509*
HAGGARD, H. W.: salivary acidity; carbohydrate- and acid-containing substances, 126
HAMILTON, T. R.: focal infection; experimental study, 481
Hamster: caries, 139, 393, 485*, 509*, 778, 844*
——: periodontal disease, 802
HARRIS, R. S.: geographic variation; cariogenic effects; riboflavin, 485*
HAWES, R. W.: ammoniated dentifrice; clinical test, 496*

HAYDON, G. B.: sodium monofluorophosphate; salivary acid; hydroxy apatite solubility, 466*
Heat injury: pulp recovery, 486*
HEDSTROM, H.: enamel permeability; fluorine, 169
——: —— ——; sugar solutions, 161
HEIN, J. W.: chlorophyll; caries inhibition, 510*
——: complex fluorides; caries inhibition, 466*
——: gingival tissues; young women, 461*
——: hamster pouchectomy; caries, 509*
——: sodium monofluorophosphate; salivary acid production; hydroxy apatite solubility, 466*
HELMAN, E. Z.: hamster caries; dietary sugar levels, 778
HENRY, E. E.: cutting efficiency; dental burs, 497*, 854
HENSCHEL, C. J.: gold castings; acid retention, 599*
Herpetic gingivo-stomatitis: aureomycin, 459*
HESS, W. C.: dentin; bound cholesterol, 837
——: ——; preservatives, 840
——: dentinal protein; recovery, 565
HILL, I. N.: fluoridation; caries experience; 12-, 13-, 14-year-olds, 465*, 670
HILL, T. J.: caries prevalence; school children, 464*
——: lactobacillus; ammonia; urea, 470*, 542
Histochemistry: gingivae, 460*, 792
Histological method: demineralization; organic chelating agents, 472*
——: electron microscopy, 476*
——: enamel matrix, 431
HODGE, H. C.: caries inhibition; complex fluorides, 466*
——: fluoride exchange; fluoride fixation, 467*
HOFFMAN, H.: lactobacilli; nucleic acids; Gram reaction, 471*
HOFFMEISTER, F. S.: malocclusion; masticatory function, 474*
HOLLAND, M. R.: local anesthetic drugs; physiological effects, 510*
HOPPERT, C. A.: albino rat caries; relative incidence, 382
HORD, A. B.: sodium fluoride; effect on dental calcium fluoride content, 360
HOROWITZ, S. L.: mandibular architecture; effect of temporalis muscle, 276
HOWARD, T. J.: caries incidence; dental students, 465*
HUNT, H. R.: albino rat caries; relative incidence, 382
HURST, V.: caries lesions; production in vitro, 482*
——: cultural characteristics; actinomycetes; lactobacilli; streptococci, 468*
——; histochemical reaction; calcium, 489*
HUTTON, W. E.: histochemical reaction; calcium, 489*
——: proteinaceous materials; enamel, 479*
HYDE, R. W.: dental disease; psychiatric patients, 806
Hypercementosis: toxic goiter, 734

IA.D.R.: executive proceedings, 524
——: index of participants, 458
——: officers elected, 530
——: presidential address, 455
——: proceedings, New York Section, November 9, 1950, 304
——: ——, ——, June 4, 1951, 599
——: ——, Pacific Northwest Section, October 22, 1950, 302
——: ——, twenty-ninth general meeting, 455
——: register; new members, 530
——: report; board of editors, 526
——: ——; publication committee, J. D. R., 527
IMHOFF, A. H.: salivary lactobacillus counts; topical fluoride applications, 251
Intercaine amide: local anesthetic, 462*
Iodoacetic acids: caries, 203
IRELAND, R. L.: Streptococcus salivarius; dental caries, 116
Ischemia: dental effects, 265
——: —— —— —— ——; mandible growth, 492*

JARABAK, J. R.: bilateral mandibular condylar resection; upper face growth, 491*
——: bone growth; alizarin, 512*
——: mandibular condyle regeneration, 491*
——: radiocalcium; alizarin; bone mineralization, 511*
——: ——; skull and face, 511*
Jaw growth: tooth development; tooth eruption; relationship, 502*
JENSEN, J.: focal infection; experimental study, 481*
JONES, E.: bone growth and repair, 487*
JUMP, E. B.: anodic decalcification; teeth, 303*

KAPLAN, H.: enamel cuticle; epithelial attachment; microstructure, 478*
KAMINS, M.: radiocalcium; skull and face, 511*
KARSHAN, M.: ovarian function; periodontosis, 460*
KAUFFMAN, S.: lactobacilli; acid production; vitamins, 470*
KELLER, R. F.: albino rat caries; relative incidence, 382
KENNEDY, D. R.: focal infection; experimental study, 481*
KILLIAN, B.: caries enamel matrix, 690
KING, R. M.: ammonium ion dentifrice; caries control, 645
——: tetradecylamine; plaque formation, 399

KITCHIN, P. C.: actinomyces; dentobacterial plaque, 88
——: salivary lactobacillus counts; topical fluoride application, 251
KLAPPER, C. E.: salivary gland extirpation; caries; hamster, 486*
KLEIN, E.: x-ray diffraction microcamera; tooth structure, 439, 476*, 478*
KNIGHTON, H. T.: oral bacteria; effect on tetanus toxin, 481*
KOSER, S. A.: lactobacilli; acid production; vitamins, 470*
KRASNOW, F.: caries incidence; increase, 512*
——: I.A.D.R., Proceedings, New York Section, November 9, 1950, 304
——: ——, ——, ——, June 4, 1951, 599*
KRAUS, E. E.: self-curing resins; evaluation, 498*
KRAUS, F. W.: saliva; antibiotic levels, 495*
KRAUS, L. L.: self-curing resins; evaluation, 498*
KRESHOVER, S. J.: pulp response; injury, 512*
KUPFER, I. J.: hypercementosis; toxic goiter, 734
KUTSCHER, A. H.: dolorimetric evaluation; dental patients, 822

LACTATION: gingival changes, 19
Lactobacilli: acid production; vitamins, 470*
——: ammonia effects, 470*, 503*, 542
——: anaerobic glycolysis; ammonia urea, 656
——: antibiotics; enzyme inhibitors, 470*
——: bacteriological procedures, 472*
——: porphyrins, 469*
——: glycolytic enzymes, 471*
——: nucleic acids; Gram reaction, 471*
——: prophyrins, 469*
——: salivary count; topical fluorides, 251
——: selective medium, 472*, 682
——: sodium azide as monilia inhibitor, 302*
LAWFORD, F. H.: saliva; ammonia production, 385
LEBERKNIGHT, A. K.: quaternary ammonium compounds; dentifrices, 558
LEOPOLD, R. S.: dentin; bound cholesterol, 837
——: ——; ——; preservatives, 840
——: ——; protein; recovery, 565
LEROY, P.: cortisone; effect on rat fetuses, 488*
——: ——; rat growth, 488*
Leukoplakia: exfoliative cytology, 260
Levo-arterenol: vasoconstriction, 462*, 870
LEVY, B. M.: mandible development; amblystoma punctatum, 490*, 575
——: vitamin B complex deficiency; mandibular joint and periodontium changes, 337
LIKENS, R. C.: fluorine adsorption; specific surface area, 467*
——: teeth; fluorine content; fluorine consumption, 172
LIND, H. E.: penicillin resistance; streptococci; staphylococci, 112
LINGHORNE, W. J.: regeneration of alveolar process, 604
Lipids: dental tissues, 599*
LISANTI, V. F.: hamster studies; caries, 139
——: carious lesions; photoelectric determination, 513*
——: pulp recovery; heat injury, 486*
LITTLE, M. F.: ammoniated dentifrices; acid production; dental plaques, 495*
——: enamel surface; chemical studies, 477*
LITWIN, T.: histochemical evaluation; gingiva, 460*, 792
——: histochemistry; gingivae, 792
LOSEE, F. L.: dentinal protein; recovery, 565
LUDWICK, W. E.: lactobacillus counts; antibiotics; enzyme inhibitors, 470*
Ludwig's Angina: carotid sinus studies, 489*
LUKS, S.: antibacterial root canal cement, 475*
LUNDQVIST, C.: iodoacetic acid; caries, 203
LUSTER, E. A.: dimensional changes agar-agar gel, 281
Lymphocytes: gingiva, 486*, 627
Lysozyme: 482*, 494*

MAHLER, D. B.: articulation change; processing dentures, 500*
MALCOLM, J.: cetylamine; toxicity, 214
Malocclusion: Class I; comparative studies, 505*
——: masticatory function, 474*
Mandible: architecture; effect of temporalis muscle, 276
——: ——; Amblystoma punctatum, 490*, 575
——: condyle; position; orthodontic treatment, 507*
——: growth; effect of condyle removal, 492*, 520*
——: physiological rest position; edentulous, 517*
——: postnatal growth; Macaca mulatta, 502*
Mandibular condyle: degeneration, 491*
——: resection; mandibular growth, 492*
—— joint: vitamin B complex effects, 337
MANHOLD, V. W.: personality; periodontal conditions, 459*
MANLY, R. S.: chewing ability; denture wearers, 314
——: ——; dentition influence, 474*
——: masticatory function; malocclusion, 474*
——: —— performance; food platform area, 475*
——: —— ——; young adults, 874
——: silicate cements; physical properties, 145

MARCUS, M.: tooth fracture; delinquency, 513*
MARSHALL, M. S.: caries lesions, production in vitro, 482*
MARSHALL-DAY, C. D.: topical fluoride; caries; adolescence, 514*
MARTIN, D. J.: fluorine content; vegetables, 466*, 676
MARTIN, E. H.: oral bacteria; effect on tetanus toxin, 481*
MASSLER, M.: dentin; amalgam action, 514*
Mastication: inadequate; compensation, 474*
Masticatory apparatus: ear disturbance, 523*
—— function: bridgework, 475*
—— ——: changing dentition, 474*
—— ——: food platform area, 475*
—— ——: malocclusion, 474*
—— performance: young adults, 874
Materials: dental; thermal conductivity, 499*
MATHIS, H.: focal infection; diagnosis, 515*
MAVROGORDATO, T.: caries; fluorine, 225
Maxilla: growth; postnatal; Macaca mulatta, 501*
Maxillary orthopedics: prosthetics, 509*
MAYNARD, E. A.: complex fluorides; caries inhibition, 466*
McBRIDE, W. D.: porphyrin compounds; effect on lactobacilli, 469*
McCLURE, F. J.: fluorine adsorption; specific surface area, 467*
——: complex fluorides; caries; bone retention, 524*
——: teeth; fluoride content, fluoride consumption, 172
MEDAK, H.: attrition; eruption and growth rate; rat incisor, 521*
MEGIRIAN, D.: fluoride exchange; fluoride fixation, 476*
Metallic salts: salivary acid production, 494*
Micrococcus lactilyticus: dentobacterial plaque, 423, 482*
—— lysodeikticus: lysozyme effect, 482*
Microstructure: enamel cuticle, 478*
——: epithelial attachment, 478*
MILLER, P. N.: silicate cements; physical properties, 145
MILLER, S. C.: cornification; oral mucosa, 4
——: glossopyrosis; vitamin B_{12}, 515*
——: prescription procedures; vitamin therapy, 516*
Mineral stress: vitamin D; parathyroids, 508*
MITCHELL, D. F.: ammoniated dentifrices; hamster caries, 393, 486*
——: ——; periodontal disease, 516*
——: hamster caries; dietary sugar levels, 778
——: periodontal disease; hamster, 802
MITCHELL, J. A.: lactobacilli; selective medium, 472*, 682
Monilia: inhibition by sodium azide, 302*
MONTGOMERY, P. W.: exfoliative cytology; leukoplakia, 260
——: ——; oral carcinoma, 308
——: ——; oral mucosa, 12
MORGAN, W. A.: ammoniated dentifrices; urea and ammonium salts content, 388
MOORE, N. A.: lactobacilli; antagonistic organisms, 468*, 665
MOORREES, C. F. A.: dentition; Aleut, 815
MORTON, H. E.: pleuropneumonia-like organisms; saliva, 415
Mucosa: oral; cornification, 4, 12
——: ——; exfoliative cytology, 4, 12
Mucoprotein: parathyroid extract, 492*
MULLINS, C. E.: caries; rat; bilateral symmetry, 62
MUNTZ, J. A.: lactobacillus; ammonia; urea, 470*, 542
MYERS, H. I.: caries incidence; dental student, 465*
——: collagenase; oral environment, 493*
——: lactobacilli; antagonistic organisms, 468*, 665

NELSEN, R. J.: restorations; evaluating adaptation, 500*
NEMES, J. L.: micrococcus lysodeikticus; lysozyme effect, 482*
——: salivary lysozyme activity; x-radiation, 494*
Nerve supply: teeth, 265
NEUWIRTH, I.: acid production; oral microorganisms; lactic and pyruvic, 100
NEVIN, R. B.: caries; physicochemical factors, 235
NEVIN, T. A.: ammoniated dentifrices; oral lactobacillus counts, 503*
——: chlorophyll; effect on oral organisms, 469*
NEWTON, M.: lipids; dental tissues, 599*
NIKIFORUK, G.: demineralization; organic; chelating agents, 472*
NIZEL, A. E.: geographic variation; cariogenic effects; riboflavin, 485*
NORRIS, T. O.: ammoniated dentifrices; urea and ammonium salts content, 388
NUCKOLLS, J.: actinomycetes; carious lesions, 517*
——: caries; enamel matrix, 690
——: ——; in vitro, 482*
——: cultural characteristics; actinomycetes; lactobacilli; streptococci, 468*
——: histochemical reaction; calcium, 489*
——: proteinaceous materials; enamel, 479*
Nucleic acids: lactobacilli, 471*
NULLETT, P.: cultural characteristics; actinomycetes; lactobacilli; streptococci, 468*
Nutrition: see specific names of vitamins and elements and diet

OARTEL, J. S.: chemical compounds; dentin effects, 477*
Occlusal planes: facial structures, 505*
O'CONNELL, B. C.: regeneration of alveolar process, 604

OGILVIE, A. L.: sodium fluoride administration; salivary gland, 516*, 712
OLECH, E.: traumatic bone cysts, 517*
OLSEN, E. S.: physiological rest position; edentulous mandible, 517*
OMATA, R. R.: fusobacteria; sensitivity; antibiotics, 799
ONISI, M.: actinomycetes; carious lesions, 518*
ORLAND, F. J.: procaine; cutaneous hypersensitivity, 462*
Orthodontic appliances; biological effect, 521*
Orthodontic treatment; mandibular condyle position, 507*
OSBORNE, D. G.: mucinous polysaccharides; streptococci, 302*
Osteosclerosis: jaws, 521*
Ovarian function: periodontosis, 460*
OVERSTREET, S.: enamel decalcification; fluorides; zinc-ferro-nitrate, 75, 189, 195, 363

PAFFENBARGER, G. C.: self-curing resins; physical properties, 497*
Pain: dolorimetric evaluation; dental patients, 822
PALMER, H. B.: enamel decalcification; fluorides; zinc-ferro-nitrate, 75, 198, 195, 363
Parathyroid: effect on blood calcium and phosphorus, 487*
——: mineral stress, 508*
——: serum microprotein, 492*
PATTERSON, T. L.: motor and secretory functions; fish stomach, 518*
——: phylogenetic evolution; teeth, 491*
PEARLMAN, S.: anaerobic glycolysis; ammonia urea, 656
——: lactobacillus; ammonia; urea, 470*, 542
Penicillin: resistance; streptococci; staphylococci, 112
Periodontal disease: ammoniated dentifrice, 516*
——: hamster, 802
——: personality, 459*
Periodontium: attachment apparatus; examinations, 638
——: cortisone, 461*
——: regeneration, 604
——: vitamin B complex effects, 337
Periodontosis: ovarian function, 460*
——: poliomyelitis, 518*, 615
PERMAR, D.: salivary lactobacillus counts; topical fluoride applications, 251
Permeability: enamel, 480*
Personality: periodontal conditions, 459*
PETRIK, L.: maxillary orthopedics; prosthetics, 509*
PEYTON, F. A.: cutting efficiency; dental burs, 497*, 854
——: dental gold wires; tensile and bending properties, 290
——: gold wires: section size; mechanical properties, 745
——: rotational speed; temperature rise, 497*, 737
pH: intraoral; micro antimony electrode, 523*
PHILLIPS, R. W.: amalgams; variations in physical properties, 499*, 845
Phosphatase: alkaline; gingiva, 28, 792
Phosphorus: blood; vitamin D; parathyroid effects, 487*
Phylogenetic evolution; teeth, 491*
Physicochemical factors: caries, 235
Plastic teeth: bonding; denture bases, 501*
Pleuropneumonia-like organisms: saliva, 415
Poliomyelitis: periodontal disease, 518*, 615
Polycarboxylic acid: 484*
Polyphosphate: decalcification, 484*
Polysaccharide: mucinous; oral bacteria, 302*, 522*
Porphyrin: effect on lactobacilli, 469*
Pregnancy: gingival changes, 19
——: nutritional states, 19
Prescription procedures: vitamin therapy, 516*
Preservatives: dentinal protein, 840
Primary teeth: caries incidence, 464*
Procaine: cutaneous hypersensitivity, 462*
Protein: dentin; cholesterol, 837
——: ——; preservatives, 840
——: ——; recovery, 565
——: enamel, 479*
Psychiatric patients; caries, 806
Pulp: blood vessels; nerve supply, 265
Pulp reaction; heat, 486*
—— ——; injury, 512*
—— ——; self-curing acrylics, 498*, 524*
Pulp removal: chromatolysis; gasserian ganglion, 496*

RACE: dentition, 815
Radiation injury: teeth, 477*, 787
Radiation: ionizing; oral effects, 33, 787
Radioactive calcium: bone mineralization, 511*
—— iodine: tooth penetration, 304*, 480*, 728
Radioactivity: teeth, 302*
Radiocalcium: dentin penetration, 304*
——: filling material; margins, 473*
——: skull and face, 511*
Radiopacity: oral structures; intraoral method, 473*
Radiographic study: physiological rest position; edentulous mandible, 517*
—— studies: jaws; sickle cell anemia, 520*
RAE, J. J.: saliva; ammonia production, 385
RAMFJORD, S.: poliomyelitis; effects on periodontium, 519*, 615
RAPP, G. W.: intermediary carbohydrate metabolism; saliva, 494*
——: intracaine amide; local anesthetic, 462*
RASMUSSON, D. F.: analysis; facial structures; occlusal planes, 505*
RATHJE, W.: sympathicotony; salivary glands; caries, 783

Regeneration: alveolar process, 604
RESCA, H. G.: salivary acidity; yeasts, 426
Resins: self-curing; chemistry, 497*
——: physical properties, 497*
——: ——; evaluation, 498*
Rest position: edentulous mandible, 517*
Riboflavin: cariogenic effect; geographic variation, 485*
RICKLES, N. H.: topical fluoride application; young adults, 519*, 757
RING, J. H.: alkaline phosphatase; healing gingival wounds, 461*
RIZZO, D. R.: lactobacillus counts; evaluation, 472*
ROBERTS, R. M.: pulp response; injury, 512*
ROBINSON, H. B. G.: actinomyces, dentobacterial plaque, 88
——: Editor's Viewpoint, 1, 157, 305, 453, 601, 753
——: I.A.D.R. Proceedings; 29th General Meeting, 455
——: salivary lactobacillus counts; topical fluoride applications, 251
——, I.B.: sickle cell anemia; jaw studies, 520*
ROGOSA, M.: lactobacilli; selective medium, 472*, 682
ROSE, S.: glossopyrosis; vitamin B_{12}, 515*
ROTH, G. D.: collagenase; oral environment, 493*
ROTH, H.: prescription procedures; vitamin therapy, 516*
Root canal cement: antibacterial, 475*
ROVELSTAD, G. H.: infectious gingivostomatitis; aureomycin, 459

SACKS, M.: enamel decalcification; fluorides; zinc-ferro-nitrate, 75, 189, 195, 363
Saliva: acid production; metallic salt effects, 494*
——: —— ——; sodium monofluorophosphate, 466*
——: acidity; carbohydrate- and acid-containing substances, 126
——: ——; yeasts, 426
——: ammonia production, 385
——: amylase; sodium fluoride activation, 599*
——: ——; temperature changes, 130
——: antibiotic levels, 495*
——: buffer; bicarbonate, 403
——: carbohydrate metabolism; intermediary, 494*
——: lysozyme activity; x-radiation, 494*
——: pleuropneumonia-like organisms, 415
——: rate; dental caries, 783
——: yeasts, 468*
Salivary glands: dental caries, 486*, 783
——: fluorides, 516*, 712
SARNAT, B. G.: mandible growth; condyle removal, 520*
——, ——: sickle cell anemia; jaw studies, 520*
——: sutural facial growth; metallic implant studies, 506*
SAVARA, B. S.: caries; incidence; primary teeth, 464*
SAVCHUCK, W. B.: fluoride; growing skeleton, 467*
SCHERP, H. W.: bacteriological studies; advancing dentinal lesions, 766
SCHLACK, C. A.: caries; rat; bilateral symmetry, 62
——: dental caries; ionizing radiation, 787
——: selenium; caries incidence, 523*
SCHMITZ, L.: levo-arterenol; vasoconstrictor activity, 462*, 870
SCHNEYER, L. H.: salivary amylase; sodium fluoride activation, 599*
——: salivary amylase; temperature effects, 130
SCHOENBAUER, F.: osteosclerosis; jaws, 521*
SCHOUR, I.: attrition; eruption and growth rate; rat incisor, 521*
SCHRAM, W. R.: bone growth and repair, 487*
SCHWARZ, M.: orthodontic appliances; biological effect, 521*
SCOTT, D. B.: enamel cuticle; epithelial attachment; microstructure, 478*
——: enamel matrix; electron microscopy, 478*
——: thin sections; electron microscopy, 476*
SCRIVENER, C. A.: fluoridation; three-year study, 465*
——: lactobacilli; antagonistic organisms, 468*, 665
SEELIG, A.: self-curing resins; pulpal response, 498*
Selenium: caries incidence, 523*
SERIO, A. F.: bondiing; plastic teeth; denture bases, 501*
SHAFER, W. G.: chlorophyll; caries inhibition, 510*
SHAPIRO, H. H.: mandibular architecture; effect of temporalis muscle, 276
——: transplantation; developing teeth, 522*
SHIERE, F. R.: hamster studies; caries, 139
——: masticatory function; dentition influence, 474*
——: streptococcus salivarius; caries, 116
SICHER, H.: traumatic bone cysts, 517*
Silicate cements: physical properties, 145
Silver nitrate: dentin effect, 477*
SIMERAL, W. G.: thermal conductivity; dental materials, 499*
SIMON, W. J.: radiocalcium; marginal adsorption; filling material, 473*
SIMMONS, N. S.: defense mechanism; oral, 494*
SIMPSON, R. L.: pulp response, 512*
SIMRING, M.: prescription procedures; vitamin therapy, 516*
SIMS, J.: caries incidence; prevalence; school children, 464*
SKINNER, E. W.: acrylic denture bases, fit, 496*
——: tooth abrasion; nylon and natural bristles, 522*

SLAWSON, M. S.: monilia inhibition; sodium azide mechanism, 302*
SMITH, F. A.: fluoride; blood, 182
——: fluorides; complex; caries inhibition, 466*
SMITH, J. D.: salivary acid production; effects of metallic salts, 494*
SMITH, P. F.: pleuropneumonia-like organisms; saliva, 415
SNYDER, M. L.: mucinous polysaccharide production; oral bacteria, 522*
——: monilia inhibition; sodium azide mechanism, 302*
——: mucinous polysaccharides; streptococci, 302*
SOBERMAN, A.: cornification; oral mucosa, 4
Sodium monofluorophosphates; salivary acid production; hydroxy apatite solubility, 466*
SOGNNAES, R. F.: enamel matrix; electron microscopy, 478*
SOYENKOFF, B. C.: lipids; dental tissues, 599*
SPRING, K. L.: ear disturbance; masticatory apparatus, 523*
SREEBNY, L. M.: collagenous activity; saliva, 493*
——: demineralization; organic chelating agents, 472*
STAHL, S. S.: cornification; oral mucosa, 4
Staphylorocci: penicillin resistance, 112
STEPHAN, R. M.: rat caries; buccal and lingual surfaces, 484*
STONEL, I. C.: cortisone acetate; periodontal tissue, 461*
STORVICK, C. A.: dental caries; Williamette Valley region, 509*
STREET, E. V.: enamel walls; cavity preparation method, 473*
Streptococci, focal infection; experimental study, 481*
——: mucinous polysaccharides, 302*
——: penicillin resistance, 112
Streptococcus salivarius: caries, 116
SUD, V.: gingival changes; deficiency; pregnancy; lactation, 19
SUESS, P. H.: enamel solubility; fluorine, 177
Sugar: dietary; caries, 778
——: enamel permeability, 161
Sulfate solutions; effects on agar-agar gel, 281

TAKATA, G. K.: tooth abrasion; nylon and natural bristles, 522*
TAPIA, C.: fluoridized enamel; acid action, 515*
TAYLOR, A. C.: denervation and ischemia; dental effects, 265
TAYLOR, B. L.: caries; rat; bilateral symmetry, 62
TAYLOR, D. F.: dental gold wires; tensile and bending properties, 290
TAYLOR, R.: ammoniated dentifrices; acid production; dental plaques, 495*
Teeth: embryology, 490*
Temperature: effects on amylase, 130
—— rise: cavity preparation; rotational speed, 487,* 737
Temporalis muscle: effects on mandible, 276
TENNENBAUM, B.: ovarian function; periodontitis, 460*
Tensile: properties; dental gold wires, 290
Tetanus toxin: effects of oral bacteria, 481*
TETER, D. E.: caries; ionizing radiation, 787
Tetradecylamine: plaque formation, 399
Thermal conductivity: dental materials, 499*
THOMAS, B. O. A.: proceedings Pacific Northwest Section, 302
——: radiation injury; teeth, 477*
THOMPSON, F. C.: micro antimony electrode; intraoral pH, 523*
THOMPSON, J. R.: bilateral mandibular condyle resection; mandible growth, 492*
——: cephalometric radiographic studies; facial anomalies, 506*
——: vertical dimension; age, 504*
THRONDSON, A. H.: levo-arterenol; vasoconstrictor activity, 462,* 870
TILDEN, E. B.: lactobacillus counts; evaluation, 472*
Torus mandibularis: Aleut, 815
Toothbrush: bristle; tooth surface abrasion, 522*
Tooth eruption; tooth development; jaw growth; relationship, 502*
Toxicity: cetylamine, 214
Toxic goiter: hypercementosis, 734
TRAUTZ, O. R.: x-ray diffraction; microcamera; tooth structure, 439, 476,* 478*
TULLIS, J. L.: ionizing radiation; oral effects, 33
TURESKY, S.: histochemical evaluation; gingiva, 460,* 792
TURNER, N. C.: viral agent; dentinal destruction, 82

UREA: ammoniated dentifrices, 388
——: anaerobic glycolysis, 656*
——: lactobacillus, 470,* 542
——: see also ammoniated dentifrices
—— solutions: endodontics, 503*
USSING, M. J.: enamel cuticle; epithelial attachment; microstructure, 478*
——: enamel matrix; electron microscopy, 478*
——: thin sections; 476*

VAN AMERONGEN, J.: caries evaluation; reproducible method, 346
Vascular supply: teeth, 265
Vasoconstriction: levo-arterenol, 462,* 870
VAUGHN, R. C.: temperature rise; rotational speed, 497,* 737
Vegetables: fluorine content, 466,* 676
VEHE, L.: alizarin; bone growth, 512*
Versene: decalcification, 484*
Vertical dimension; occlusal rest; age, 504*
VILLA, V. G.: enamel matrix; histologic technique, 431

VINTON, P. P.: chewing ability; denture wearers, 314
Virus: dentinal destruction, 82
Vitamin B complex: mandibular joint; periodontium, 337
Vitamin B_{12}: glossopyrosis, 515*
—— D: effect on blood calcium and phosphorus, 487*
—— D: mineral stress, 508*
—— E: enamel depigmentation, 508
—— lactobacilli: acid production, 470*
—— therapy: prescription procedures, 516*
VOLKER, J. F.: caries production; hamster; age, 484*
VON HAAM, E.: exfoliative cytology; oral carcinoma, 308
——: ——; leukoplakiia, 260

WACHTL, C.: enamel; permeability, 480*
WAH LEUNG, S.: bicarbonate; salivary buffer effect, 403
WAINWRIGHT, W. W.: dentin permeability; radioactive materials, 480*
WALSH, J. P.: caries; physicochemical factors, 235
——: enamel decalcification; surface active agent, 218
WARNER, B. W.: caries incidence; dental students, 465*
——: lactobacilli; antagonistic organisms, 468,* 665
WEINMANN, J. P.: traumatic bone cysts, 517*
WEISENGREEN, H. H.: Ludwig's angina; carotid sinus studies, 489*
WELCH, F. E.: silicate cements; physical properties, 145
WHEATCROFT, M. G.: caries; rat; bilateral symmetry, 62
——: micrococcus lysodeikticus; lysozyme effect, 482*
——; salivary lysozyme activity; x-radiation, 494*
——: selenium; caries incidence, 523*
WILLIAMS, N. B.: pleuropneumonia-like organisms; saliiva, 415
WINKLER, K. C.: caries evaluation; reproducible method, 346
WISEMAN, R. F.: lactobacilli; selective medium, 472,* 682
WOLCOTT, R. B.: restorations; evaluating adaptation, 500*
WOLF, W.: fluoridation; caries experience; 12-, 13-, 14-year-olds, 465,* 670
WUEHRMANN, A. H.: radiopacity; intraoral method, 473*
WYCKOFF, R. W. G.: enamel matrix; electron microscopy, 478*
——: thin sections; electron microscopy, 476*

X-RAY diffraction; microcamera; tooth structure, 439, 476,* 478*
——: rodent enamel, 828
——: edentulous jaws, 504*
——: oral effects, 33
——: radiation: 494*
——: radiopacity, 473*
X-radiation: salivary lysozyme activity, 494*

YEASTS: saliva, 468*
——: salivary acidity, 426
YOUNG, G.: salivary acidity; yeasts, 426
YURKSTAS, A.: carious lesions; photoelectric determination, 513*
——: functional evaluation; bridgework, 475*
——: inadequate mastication; compensation, 474*

ZANDER, H. A.: hamster studies; caries, 139
——: penicillin resistance; streptococci; staphylococci, 112
——: pulp reaction; resinous filling materials, 524*
——: —— recovery; heat injury, 486*
ZEIDENSTEIN, S.: labial and buccal glands; fetal origin, 587
ZIMMERMAN, A. A.: labial and buccal glands; fetal origin, 587
Zinc-ferro-nitrate; enamel decalcification, 75, 189, 195
——: dentin effects, 477*
ZIPKIN, I.: decalcification versene polycarboxylic acids; polyphosphates, 484*
ZIPKIN, I.: complex fluorides; caries; bone retention, 524*
ZWEMER, E.: fluoridized enamel; acid action, 515*
ZWIRNER, E.: alveolo-dental joint; examination technique, 638

CONTENTS FOR DECEMBER, 1951 (Cont'd)

In Vitro Sensitivity of Fusobacteria to Various Antibiotics. Robert R. Omata. (National Institute of Dental Research, Bethesda, Md.) ---------- 799

Two Studies of Periodontal Disease in the Syrian Hamster. David F. Mitchell and D. S. Cheranusek. (University of Minnesota, Minneapolis, Minn.) -------- 802

Dental Disease in Psychiatric Patients. James M. Dunning, Robert W. Hyde, and Peter J. Dalton. (Boston Psychopathic Hospital, Boston, Mass.) ---------- 806

The Dentition as a Criterion of Race With Special Reference to the Aleut. Coenraad F. A. Moorrees; Tandarts, D.D.S. (Forsyth Dental Infirmary for Children, Boston, Mass.) ---------- 815

Dolorimetric Evaluation of Dental Patients. Austin H. Kutscher, B.A., D.D.S., and Neal W. Chilton, B.S., D.D.S., M.P.H. (Columbia University, N. Y.) -- 822

Studies on the Physical Properties of Rodent Enamel. II. Density Distribution and X-Ray Diffraction Pattern. J. Edward Gilda. (The University of Rochester, Rochester, N. Y.) ---------- 828

Dentinal Protein: Bound Cholesterol. R. S. Leopold, Ph.D., W. C. Hess, Ph.D., and W. J. Carter, D.D.S. (Georgetown University, Washington, D. C.) ---- 837

Dentinal Protein: Some Effects of Preservatives. W. J. Carter, D.D.S., R. S. Leopold, Ph.D., and W. C. Hess, Ph.D. (Georgetown University, Washington, D. C.) ---------- 840

Physical Properties of Amalgam as Influenced by Variation in Surface Area of the Alloy Particles. Walter S. Crowell and Ralph W. Phillips. (Indiana University School of Dentistry, Indianapolis, Ind.) ---------- 845

A Study in the Cutting Efficiency of Dental Burs for the Straight Handpiece. E. E. Henry, M.S.E., and F. A. Peyton, D.Sc. (School of Dentistry, University of Michigan, Ann Arbor, Mich.) ---------- 854

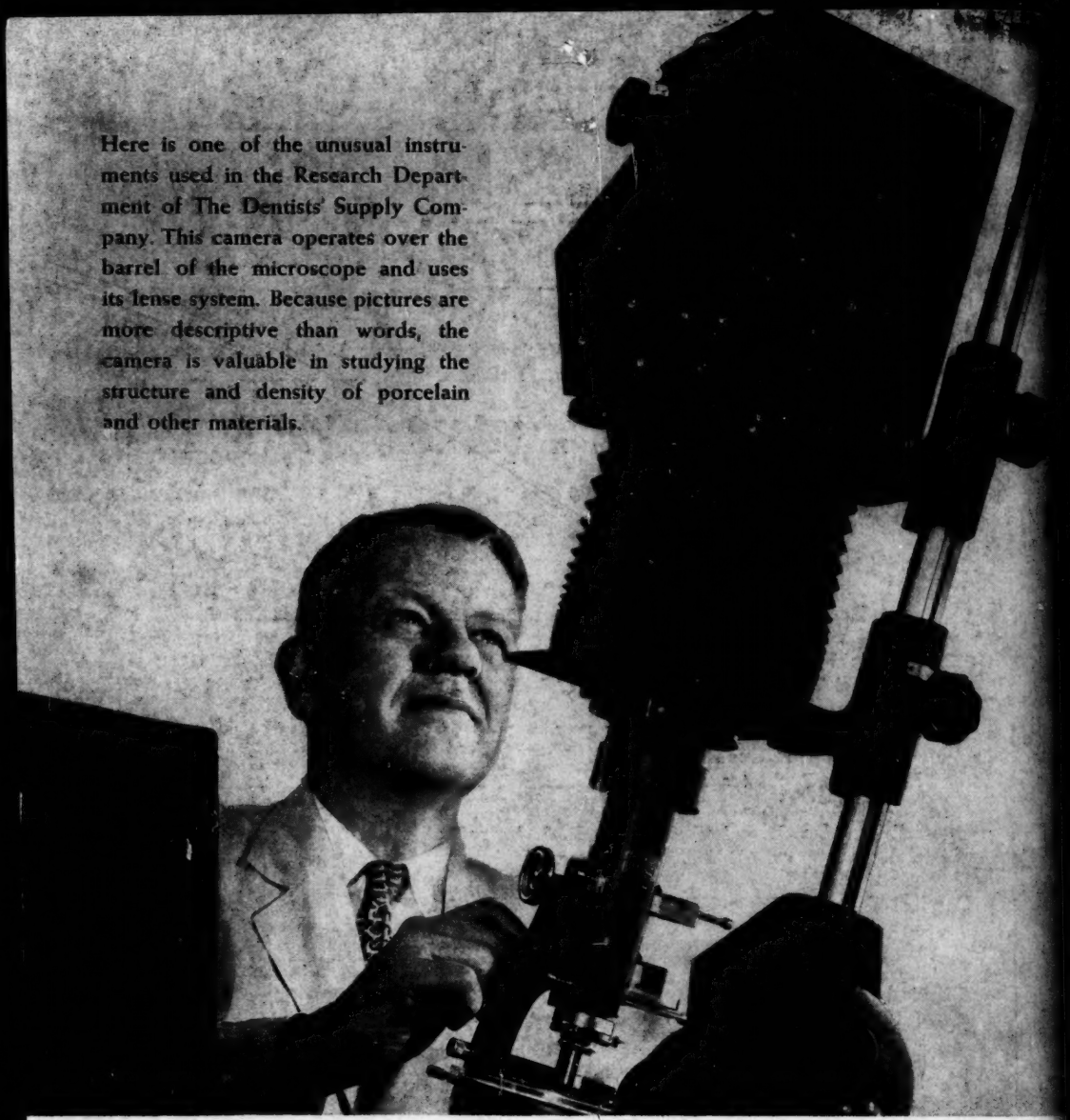
Levo-Arterenol (Levophed) as a Vasoconstrictor in Local Anesthetic Solutions. Sidney Epstein, D.D.S., Albert H. Throndson, D.D.S., and J. Leonard Schmitz, D.D.S. (College of Physicians and Surgeons, School of Dentistry, San Francisco, Calif.) ---------- 870

Factors Affecting Masticatory Performance and Efficiency Among Young Adults. R. S. Manly. (Tufts College Dental School, Boston, Mass.) ---------- 874

International Association for Dental Research Program of the Chicago Section for the Year 1950-1951. Compiled by Frank Orland, Editor, Chicago Section ---- 883

Index to Volume 30 ---------- 885

Contents to Volume 30 ---------- iii



Here is one of the unusual instruments used in the Research Department of The Dentists' Supply Company. This camera operates over the barrel of the microscope and uses its lense system. Because pictures are more descriptive than words, the camera is valuable in studying the structure and density of porcelain and other materials.

"DENTSPLY" IS STILL PIONEERING IMPROVEMENTS IN ARTIFICIAL TEETH

Many of the developments that are now so commonplace in prosthetic work had their beginnings in the achievements of our Research Department. Our recent development of Trubyte Dentron Teeth emphasizes the determination of The Dentists' Supply Company to provide you with the best in artificial teeth—whether porcelain or plastic.

TRUBYTE **Dentron®** TEETH

THE PLASTIC TEETH WITH 10 ADVANTAGES

ANOTHER RELIABLE PRODUCT OF DENTSPLY RESEARCH